

**Physiological and whole-body correlates of contest  
behaviour in the hermit crab *Pagurus bernhardus***

By

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A thesis submitted to the University of Plymouth  
in partial fulfilment for the degree of

DOCTOR OF PHILOSOPHY

School of Biological Sciences  
Faculty of Science

May 2009

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# **Physiological and whole-body correlates of contest behaviour in the hermit crab *Pagurus bernhardus***

**By Sophie Mowles**

**Abstract.** The series of experiments that comprise this thesis used shell fights in the hermit crab *Pagurus bernhardus* as a model system to address the proximate physiological and whole-organism correlates of contest behaviour. These correlates of fighting ability ranged from the metal ions magnesium ( $Mg^{2+}$ ) and calcium ( $Ca^{2+}$ ), through respiratory pigments and muscular proteins to whole-organism performance capacities and behavioural syndromes. This broad-spectrum approach thus allowed the study of a suite of mechanisms which may influence agonistic behaviour and fighting ability. The winners of shell fights were found to have higher levels of  $Mg^{2+}$  and  $Ca^{2+}$  than losers, and it is likely that increased concentrations of these metal ions may enhance the oxygen-affinity of the crustacean respiratory pigment haemocyanin (Hc). Furthermore, it was found that winners had higher concentrations of Hc than losers. Thus, aerobic capacity is important to fighting hermit crabs and its enhancement may increase the stamina of competitors by delaying the onset of anaerobic respiration. Stamina is assumed by models such as the energetic war of attrition to be correlated with agonistic success. In agreement with this assumption, an experiment utilising performance capacities to obtain a measure of stamina found that crabs with greater performance capacities were more likely to win shell fights. In analysing muscle quality as a proxy for strength, it was found that winners had higher muscle/body weight ratios than losers, although there was no difference in the muscle protein concentrations between the fight outcomes. Finally, behavioural syndromes were identified in *P. bernhardus*, although these

did not influence fighting ability whereas aerobic capacity did. Thus, despite an individual's behavioural type, it is its performance capabilities that dictate whether it will succeed in fights. The work presented here thus demonstrates that a suite of physiological and whole-organism variables influence contest behaviour in hermit crabs, and that both aerobic and performance capacities are very important in determining agonistic success.

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## Acknowledgements

I am grateful to the University of Plymouth for providing the funding for this work *via* a PhD studentship.

I would first and foremost like to thank Mark Briffa and Pete Cotton for their supervision and support over the course of my PhD. They have provided me with advice and encouragement, as well as a lot of flexibility, allowing me the scope to develop an interesting and enjoyable sequence of experiments. Thanks to them for being excellent and good humoured supervisors, and for occasionally helping me out in the field when it was a good low tide and wasn't too rainy.

Thanks to John Spicer for advice on physiological mechanisms, for providing entertainment on the Portugal field courses, and for mentioning the word 'magnesium' on a cold and rainy mudflat in the Algarve when desperately looking for *Uca*. It all started there. Also thanks to Kath Sloman for assistance with the Bradford Protein Assay.

I would like to thank Richard Thomson, Pete Cotton and Mark Briffa for the opportunity to demonstrate on the local and residential field courses, which were great fun. Also thanks to Ana Silva for linguistic advice and introducing me to various 'edible' marine organisms. The baby *Sepia* were nice, but I'm still not so sure about the *Patella* sashimi.

Thanks to all the Andys for technical support: Andy Arnold, Andy Fisher, Andy Atfield and Andrew Tonkin, as well as the nominal non-conformists Ann Torr, Nick Crocker and Richard Ticehurst.

Also, thanks to all my friends and colleagues on the 6<sup>th</sup> floor, especially Emma Sheehan, Sally Marsh, Samantha Fowell, Emma Jackson, Genoveva Gonzalez Mirelis, John Griffin, Kate De La Haye and everyone else that there just isn't room here for.

I would also like to thank Tim Dickinson for his support throughout the last parts of this PhD, and for providing a different perspective on things (artistic *versus* scientific), as well as his refreshingly unscientific and unbiased interest in the natural world.

Last, but not least, I'd like to thank my family for their support, especially my parents, Graham and Glynis Mowles for financial support for the degree that got me to this point. Thanks also to Samantha Mowles, Sarah, Karl, and Natalie Vosper. Also, thanks to my Godmother, Eve Eggington, whose idea it was to buy me comprehensive books on insects as a little girl and probably started this whole business in the first place.

## **Author's Declaration**

At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate committee.

This study was financed with the aid of a studentship from the University of Plymouth.

Relevant scientific conferences were regularly attended at which work was often presented.

### **Publications:**

Briffa, M. & Mowles, S. L. Hermit crabs. *Current Biology*, **18**, r144-r146.

Mowles, S. L., Briffa, M., Cotton, P. A. & Spicer, J. I. The role of circulating metal ions during shell fights in the hermit crab *Pagurus bernhardus*. *Ethology*, **114**, 1014-1022.

### **Conference participation and presentations:**

Mowles, S. L., Briffa, M. & Cotton, P. A. Effects of respiratory pigment and whole body performance capacity on agonistic signals in the hermit crab *Pagurus bernhardus*. ISBE 2008: 12<sup>th</sup> International Behavioural Ecology Congress. Cornell University, Ithaca, New York, USA. 9-15 August, 2008. Oral presentation.

Mowles, S. L., Briffa, M., Cotton, P. A. & Spicer, J. I. Effects of metal ions and respiratory pigment during agonistic encounters in the hermit crab *Pagurus bernhardus*. Marine Institute Conference: Understanding Marine Systems. University of Plymouth, Plymouth, Devon, UK. 17<sup>th</sup> December 2007. Oral presentation.



Mowles, S. L., Briffa, M. Cotton, P. A. & Spicer, J.I. Champions how their mettle! Seasonal effects of metal ions on contest behaviour in the hermit crab *Pagurus bernhardus*. IEC 2007: XXX International Ethological Conference. Dalhousie University, Halifax, Nova Scotia, Canada. 15-23 August 2007. Poster presentation.

Mowles, S. L., Briffa, M. Cotton, P. A. & Spicer, J.I. The effect of respiratory pigment on shell fighting in the hermit crab *Pagurus bernhardus*. IEC 2007: XXX International Ethological Conference. Dalhousie University, Halifax, Nova Scotia, Canada. 15-23 August 2007. Poster presentation.

Mowles, S. L., Briffa, M. Cotton, P. A. & Spicer, J.I. The role of circulating metal ions during contest behaviour in hermit crabs. ASAB Easter meeting. University of Exeter, Cornwall campus, Falmouth, UK. 28-30 March 2007. Oral presentation.

Mowles, S. L., Briffa, M. Cotton, P. A. & Spicer, J.I. The role of circulating metal ions in decision-making during shell fights in the hermit crab *Pagurus bernhardus*. Synergy between the University of Exeter and the University of Plymouth. University of Plymouth, Plymouth, Devon, UK. 11<sup>th</sup> December 2006. Oral presentation.

Mowles, S. L., Briffa, M. Cotton, P. A. & Spicer, J.I. Champions how their mettle! Seasonal effects of metal ions on contest behaviour in the hermit crab *Pagurus bernhardus*. ISBE 2007: 11<sup>th</sup> International Behavioural Ecology Congress. Tours, France. 23-29 July 2006. Poster presentation.

Word count of main body of thesis: 41,074

Signed.....  
Date.....22/05/2009.....

# CHAPTER 1

## Introduction

## ***Abstract***

Animal contests involve a series of decisions that are based upon the value of the contested resource, as well as the relative or absolute fighting ability of the contestants. Dangerous fighting does occur in nature, although it is usually avoided in agonistic interactions, with encounters usually being resolved by stereotyped fight displays, which may permit mutual assessment of resource holding potential (RHP). Game theoretical analyses have illustrated how these “limited war” strategies are the predicted result of natural selection. However, with the evolution of mutual assessment comes the possibility of cheating, whereby an animal may signal that it is of better quality than it actually is. Signalling systems are therefore predicted to be costly to restrict invasion by such cheats. Intrinsic costs associated with the production of many signals constrain the level of their production and thus preclude animals from signalling at levels beyond their capability. A recent development in the study of contest behaviour has been the analysis of fights with respect to the physiological consequences of engaging, such as energetic costs, and endocrine changes. Thus the investigation of proximate mechanisms that potentially constrain and control demanding activity can provide insight into how strategic decisions may be made during contests.

## ***Contests***

In nature, resources are generally less abundant than required by the competitors that seek to gain access to them, and so competition is predicted to occur over resource ownership. This can either take the form of scramble competition, or contests between two opponents. When the value of the resources (‘resource value’, RV) increases, either because they are particularly scarce, or because they yield higher fitness gains, aggression is usually more intense. The intensity of contests can also depend on the relative resource holding potential (RHP; Parker 1974) of the opponents, with the intensity of fighting increasing as the difference in relative RHP decreases (see Briffa and Elwood *In Press*; Gammell & Hardy 2003; Taylor & Elwood 2003). The RV and RHP of opponents vary such that, although a common component of biological systems, contest behaviour manifests itself in a diversity of ways.

One way in which animal aggression can occur is in the form of dangerous fighting. This can result in serious injury to the individuals that engage in it, and the literature includes many examples of species where injury or death occurs in a relatively high percentage of

competing individuals. For example, three species of ungulate are frequently cited as contest systems where injuries may be sustained during the course of aggressive encounters. Clutton-Brock et al. (1979) observed that up to 6% of rutting red deer (*Cervus elaphus*) stags are permanently injured each year, amounting to approximately 20-30% sustaining permanent injury during their lifetime. Similarly, Geist (1974) observed that up to 10% of male mule deer (*Odocoileus hemionus*) of more than 1.5 years of age show some sign of injury each year. Even more extreme casualties are sustained by adult bull musk oxen (*Ovibos moschatus*), 5-10% of which may die each year from rutting injuries (Wilkinson & Shank 1977).

Although an injury may not be immediately life threatening, it may still affect the fitness of the individual. This could be through altering future fight performance or by increasing the individual's vulnerability to predation (Clutton-Brock et al. 1979), or its ability to attract mates. As the weaker members of populations are those most likely to be targeted by predators, an injury sustained in a contest may be life threatening, even if not initially serious. Given the risks involved with dangerous fighting, it is not surprising that fights are either settled, or preceded, by stereotyped displays that may permit mutual assessment.

Such non-injurious conflict can take the form of agonistic displays (e.g. Payne & Pagel 1996; Payne & Pagel 1997) or trials of strength (Clutton-Brock & Albon 1979), which allow the contestants to assess their relative RHP without actually risking harm. Maynard Smith and Price (1973) referred to such contests as being of a "limited war" type, involving "inefficient weapons or ritualised tactics that seldom cause injury to either contestant". The original explanation for such restrained contest behaviour was that it was the product of group selection, as the injury of many individuals "would militate against the survival of the species" (Huxley 1966), although this idea is now discredited. An alternative theory was proposed by Hamilton (1971), who emphasized that aggressive animals would run the risk of injuring their close relatives, thereby reducing their inclusive fitness. Thus, kin selection has also been used to explain the rarity of dangerous fighting. However, in 1973 Maynard Smith & Price proposed a model for the evolution of contest behaviour that was based on frequency-dependent natural selection. Here selection acts at the level of the individual, but the success of the strategy adopted by the individual depends on the strategies adopted by the other members of the population. They believed that group selection alone could not be responsible for the complex array of anatomical and behavioural adaptations for conventional fighting found in so many species, and that selection must be acting at the level of the

individual for these effects to be produced. This has been predicted through the development of evolutionary game theory.

### ***Game Theory***

Game theory was originally developed for use in human economic and conflict behaviour (Nash 1951; Neumann & Morgenstern 1944). However, there is difficulty in assigning a numerical value (“utility”) on the values that the players place on the possible outcomes. This is because humans think in terms of appeasement, meaning that utilities such as employment and financial reward end up being placed on the same numerical scale as more serious matters such as injury and death (Maynard Smith 1974).

In contrast, evolutionary game theory centres on the relatively simple “desires” of non-human animals – maximising their fitness, and selecting the appropriate strategy in order to do this. Unlike humans, these ecological “choices” are not conscious decisions of the part of the players, but are traits that have been naturally-selected in order to maximise the organism’s fitness (Maynard Smith 1974). Although Lewontin (1961) made the original attempt to apply the theory of games to evolution, it was Maynard Smith and colleagues who applied it to animal conflicts (Maynard Smith 1974; Maynard Smith & Parker 1976; Maynard Smith & Price 1973), leading to our present understanding of the evolution of contest behaviour.

The basic concept of evolutionary game theory is to model ecological situations as strategic games, where alternative strategies result in payoffs, which can be expressed numerically via mathematical models. These payoffs are derived from the costs of fighting ( $C$ ) and the value of the resource ( $V$ ), but are ultimately dependent on what other members of the population are doing, resulting in frequency-dependent selection as described by Maynard Smith & Price (1973). The solution to such games is described as an “evolutionarily stable strategy” or ESS. This is defined as a strategy that, if adopted by all of the members of a population, could not be invaded by a “mutant” that adopted an alternative strategy (Maynard Smith & Parker 1976; Maynard Smith & Price 1973).

Maynard Smith & Price (1973) stressed that a “mixed” ESS may exist where the individuals present adopt one of a set of “pure” strategies. Here, a stable population could either be genetically polymorphic, with individuals permanently adopting different pure strategies; or monomorphic, where the individuals switch between playing each strategy. In

both cases, the behaviours would be expressed in the frequencies appropriate to maintaining a stable strategy (Maynard Smith 1974).

In calculating the ESS, costs ( $C$ ) represent the expenditure of energy and time, as well as the more serious disadvantage of being injured (Maynard Smith & Price 1973). Value ( $V$ ), however, is not measured by the immediate fitness gain provided by acquiring the contested resource, but by the energy that the loser must expend in securing a second, similar resource (Maynard Smith 1974). Maynard Smith & Price (1973) used these factors to explain how animals should act in contest situations, and whether total or limited war should be favoured depending on the values of  $V$  and  $C$ . They developed what is widely known as “The Hawk-Dove Game” (*sensu* Maynard Smith & Parker 1976), which predicted that dangerous fighting is only an ESS when  $C < V$ .

The Hawk-Dove game is a useful tool in understanding how conventional fighting is predicted by selection when  $V < C$ . However, it is simplified in the extreme. In nature, contests are not single iterative events as in the Hawk-Dove game, but may be composed of many bouts of repetitive or escalatory behaviours (e.g. Briffa & Elwood 2000b; Briffa et al. 1998; Burmeister et al. 2002; Clutton-Brock & Albon 1979). Despite the simplicity of such models, they are useful tools in demonstrating the underlying reasons for why animals may adopt particular behaviours in contest situations. For example, the relative values of  $V$  and  $C$ , when expressed in a model can help one understand certain aspects of red deer contest behaviour. Clutton-Brock et al. (1979) described how stags fight harder when potential benefits ( $V$ ) are high, but tend to avoid fighting when they are unlikely to beat their opponent (potential  $C$ ). This presents another aspect of contest behaviour: assessing one’s opponent and then modifying the strategy used accordingly.

## ***Assessment***

The evolution of the assessment of relative fighting ability is predicted to the same extent as the ability to inflict costs on one’s opponent. The Hawk-Dove game, as presented by Maynard Smith & Price (1973) shows that while Hawks are superior in fights against Doves, if faced by a Hawk, Dove is the better strategy to play in order to avoid the costs of injury. It would therefore be advantageous to be able to assess these qualities in an opponent before deciding on the strategy to adopt in the current contest. In their modified Hawk-Dove-Assessor game, Maynard Smith & Parker (1976) revealed that the Assessor strategy is an

ESS. Here, Assessors gain information about their opponents' fighting ability and retreat from Hawks, but escalate against Doves, resulting in the highest fitness payoffs of the three strategies. In nature, the process of assessment can take a variety of forms, usually occurring as either trials of strength (Parker 1974), or as agonistic signals, which can either be 'conventional' or 'costly'.

### ***Honesty and Bluffing***

With the evolution of mutual assessment comes the possibility of benefiting by cheating, where an individual signals that they are of better quality than they actually are. Low levels of cheating are predicted in naturally occurring populations because of the fitness benefits awarded to cheating individuals (Dawkins & Guilford 1991). However, the benefits derived from cheating are frequency dependent, as high proportions of cheats would favour the evolution of more discriminate assessors. Thus, signals are generally honest and cheating remains present at low levels. Cheating is dependent on the mutual allocation of costs during information transfer, whereby both the signaller and receiver pay a cost as a result of the signal (Dawkins & Guilford 1991). Here, signallers benefit from producing signals that are cheap to perform while receivers benefit from accepting signals that are cheap to receive. The costs to receivers are largely time costs, and they benefit from assessing a signal that provides them with a quick but reliable estimate of signaller ability rather than its full potential. Such signals are 'conventional' signals, where a trait such as body size or ornamentation is used as an index of quality. However, such visual cues may not give an accurate estimate of the RHP of the opponent. For example, large individuals may not win because they may be in poor condition due factors such as age, illness, or exhaustion. Thus conventional signals are open to 'cheating' (Dawkins & Guilford 1991). For example, male green frogs can lower the pitch of their calls, potentially exaggerating their RHP as dominant frequency is negatively correlated with body size (Bee et al. 2000). There are also notable examples where organisms have been demonstrated to be capable of "bluffing" their ability, the majority of these being crustaceans. Grafen (1990) defines bluff as the unintentional exaggeration of advertised fighting ability. This is best exemplified in newly moulted stomatopods (Adams & Caldwell 1990; Steger & Caldwell 1983); fiddler crabs with regenerated claws (Backwell et al. 2000); and snapping shrimps, where weapon (chela) size does not perfectly correlate with body size (Hughes 2000). Thus, some crustaceans are able to "bluff" by communicating a different

resource holding potential to their true state via a visual signal consistent with conventional signalling. Cheating, however, remains at low levels within a population as this strategy is not an ESS. High levels of bluffing cannot exist as the information conveyed would have little or no value to receivers, and would thus be ignored. Furthermore, there may costs associated with retaliation from the receiver if the false signal is discovered or a genuinely strong receiver ‘calls the bluff’ of the sender (Számadó 2008).

Costly signals however, are not generally vulnerable to invasion by cheats (Grafen 1990; Zahavi 1977). Here, an intrinsic cost associated with the production of the signal constrains the level of its performance and thus precludes an animal from signalling at a level beyond its capability. The signals usually involve the performance of a potentially exhausting activity (Briffa & Elwood 2001; Clutton-Brock & Albon 1979), and thus accurately reveal the quality of the signaller once it reaches its maximum signalling capacity (Grafen 1990). This is one of the explanations for the evolution of repeated displays as seen in many signalling systems, such as roaring in red deer (Clutton-Brock & Albon 1979) and shell-rapping in hermit crabs, *Pagurus bernhardus* (Briffa 2006; Briffa & Elwood 2000b; Briffa et al. 1998).

### ***Repeated Signals***

Clutton-Brock & Albon (1979) first hypothesized that competitors may assess each other’s fighting ability using the rates and duration of agonistic displays, which would explain the performance of repetitive stereotyped movements. However, Enquist et al. (1990) proposed that such repetitious displays are a result of assessment being a gradual process. This is supported by an increase in fight duration as the asymmetry between opponents decreases. Thus there may be different reasons behind the repetitive nature of some signals; Payne & Pagel (1997) highlight three mechanisms for this. They propose that displays may be repeated either: to confirm the level of the signal; to replace previous signals; or to augment previous signals.

The first reason for repetition presented by Payne & Pagel (1997) is equivalent to the ‘Sequential Assessment Model’ (SAM) (Enquist 1985; Enquist & Leimar 1983; Enquist et al. 1990; Leimar et al. 1991; Leimar & Enquist 1984). This model assumes that information is gained throughout the course of a display in a way analogous to statistical sampling, such that each repetition enhances the accuracy of the receiver’s assessment of the signaller. Enquist et



al. (1990) have empirically tested this model using cichlid fish (*Nannacara anomala*), which exhibit escalatory fighting behaviours that increase in intensity if the asymmetry between opponents is decreased. Thus, in order to gain a more accurate representation of an opponent's ability, increasingly costly behaviours must be employed during the contest.

The second 'reason' for repetitive signalling presented by Payne & Pagel (1997) is that of replacement. This model assumes a small amount of dishonesty but in this case by way of 'false modesty', rather than an exaggeration of RHP. Contestants initially perform low-level, low-cost signals, but escalate if their opponent signals an ability greater than theirs, and they are able to respond with a greater signal. This 'best-so-far' rule has been demonstrated by Wagner (1992) using cricket frogs, which respond only to the largest signal generated during the contest.

The third 'reason' for repeating displays is to augment the overall signal, as exemplified in the model for displays of endurance presented by Payne & Pagel (1996). This model is termed the 'Energetic War of Attrition' (E-WOA), and each repetition contributes to the overall signal, which provides an indication of stamina. However, this model does not involve mutual assessment as the decision to withdraw is made once the signaller triggers its own energetic cost threshold. Some of the best known examples of animals utilising the E-WOA for assessment are red deer roaring (Clutton-Brock & Albon 1979) and hermit crabs shell-rapping (Briffa & Elwood 2000b). A development of this model is the 'cumulative assessment model' (CAM: Payne 1998), where in addition to the costs of performance, contestants are subject to costs inflicted directly by the opponent. Again, this is a 'cumulative' model where giving up is based on an individual cost threshold, in the absence of mutual assessment.

### *Signals of stamina*

Signals of stamina are assumed by the E-WOA and CAM, and have been supported by empirical studies which show that the vigour of agonistic displays is correlated to the stamina of the sender (e.g. Brandt 2003; Leal 1999) and with agonistic success over a wide range of taxa including hermit crabs (Briffa et al. 1998); fish (see Abrahams et al. 2005); toads (Sullivan & Walsberg 1985); lizards (Molina-Borja et al. 1998), birds (Weary et al. 1991) and red deer (Clutton-Brock & Albon 1979).

Many studies analyse contests with respect to their physiological consequences in order to measure the costliness of signalling. These costs may be endocrine changes (Briffa & Elwood 2007; Sneddon et al. 2000b), or energetic costs associated with producing the signal, including the accumulation of metabolic by-products such as *L*-lactate (Briffa & Elwood 2001; Briffa & Elwood 2005; Neat et al. 1998; Schuett & Grober 2000; Sneddon et al. 1999; Thorpe et al. 1995), and the depletion of energy reserves such as glycogen (Briffa & Elwood 2001; Briffa & Elwood 2004; Neat et al. 1998; Sneddon et al. 1999; Thorpe et al. 1995). Thus, the metabolic consequences of performing demanding signals may impose physiological limitations to behaviour, thereby precluding an animal from signalling beyond its capacity (Hurd 1997). Although such physiological consequences of demanding agonistic activity have been analysed in a range of taxa (see above examples), a well-established model system for the investigation of contest behaviour, repetitive signalling and its physiological correlates is shell fighting in the hermit crab *Pagurus bernhardus*.

### ***Hermit crabs***

Hermit crabs are Anomuran decapod crustaceans, and are thus more closely related to stone and king crabs (Lithodidae), squat lobsters (Chirostylidae, Galatheididae) and porcelain crabs (Porcellanidae) than to the true Brachyuran crabs. However, hermit crabs are unusual amongst the Anomura as most species have an asymmetrical body plan and a curved membranous abdomen. In the absence of a calcified exoskeleton on the abdomen, hermit crabs occupy hollow objects, typically gastropod shells, in order to provide a degree of buffering against environmental fluctuations and protection from predatory attack (see Briffa & Mowles 2008). These hollow objects are generally carried around by the hermit crab, being likened to ‘portable burrows’, although some sedentary species do exist, living in structures such as the empty tubes of coral-dwelling sabellid polychaete worms, which they rarely leave (Gherardi 1996).

There are five distinct families of hermit crabs: the Pylochelidae (symmetrical hermit crabs), Paguridae (right-handed species), Diogenidae (left-handed species), Parapaguridae (deep-sea species), and Coenobitidae (terrestrial species). Most of these families possess the curvature of the abdomen and asymmetrically-sized chelipeds which are adaptations to occupying empty gastropod shells. The curvature of the abdomen and modified uropods allow the hermit crab to fit into, and grip onto the spiral of the gastropod shell. The larger of

the two chelipeds covers the aperture of the shell when the crab is fully withdrawn, analogous to an operculum. The exception to this is the coconut crab *Birgus latro*, which as an adult becomes completely terrestrial with the development of a pseudo-lung and a fully calcified abdomen coiled underneath the cephalothorax similar to true crabs (Debelius 2001). However, juvenile coconut crabs occupy gastropod shells, similar to other hermit crabs. As shell-occupying hermit crabs occupy a progression of shells of increasing size increments throughout their ontogeny (see Elwood & Neil 1992), and as coconut crabs are capable of reaching sizes of up to 5 kg, this transformation presumably occurs once suitable shells can no longer be found (Debelius 2001). In contrast however, typical shell-occupying hermit crabs must find a new suitably sized gastropod shell every time that their size increases. Thus, the size and weight of hermit crabs are positively related to the size and weight of the shells that they occupy (Briffa & Elwood 2007).

The suitability of shells varies not only with their size, but also in terms of the species and quality of the shell. For example, the hermit crab *P. bernhardus* has a known preference for *Littorina littorea* over *Gibbula umbilicalis* or *G. cineraria* shells (Dowds & Elwood 1983), although this preference changes with the size of the crabs. Small *P. bernhardus* tend to occupy *L. obtusata* shells, progressing to *Gibbula* spp., *Nucella lapillus*, *L. littorea* and finally to *Buccinum undatum* as their size increases (see Elwood & Neil). Shell suitability is further influenced by the quality of the shell; and shells with physical damage, or internal volumes that are too small for the size of the occupying hermit crab are of poor quality, increasing the risk of predation as well as reducing growth rate and fecundity (Dowds & Elwood 1983). Obtaining a shell of good quality is thus very important to hermit crabs.

### ***Contests in hermit crabs***

Suitable shells are often in short supply, and hermit crabs must generally fight in order to take good quality shells from other hermit crabs. The majority of research on this subject has been carried out using *P. bernhardus* (but see Gherardi 2004; Hazlett 1996). The contestants adopt two very different roles during the encounter, which are decided following a period of 'pre-fight displays' (Arnott & Elwood 2007; Elwood et al. 2006). Such displays involve the presentation and extension of the chelipeds, which are assumed to demonstrate the size of the opponents prior to engaging in a shell fight. Following this, the larger crab usually initiates the fight, becoming the 'attacker'. This individual then attempts to take the shell of the non-

initiator or 'defender' following an assessment period comprising bouts of 'shell-rapping', during which the defender remains tightly withdrawn inside its shell. The attacker, having first overpowered the defender, grips the shell of the defender and proceeds to bring its own shell repeatedly into contact with that of the defender as a bout of raps in quick succession. A series of such bouts, separated by pauses, is performed during the shell fight. The vigour of rapping, and therefore the advertised fighting ability of the attacker (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998; Elwood & Neil 1992), varies in terms of the number of raps in each bout, the duration of the pauses between bouts (Elwood & Briffa 2001), and the force of the raps (Briffa & Elwood 2002). Shell fights can end in one of two ways, either the defender decides to give up; allowing itself to be evicted by the attacker, which removes the defender through the aperture of the shell; or the attacker may decide to give up first without having evicted the defender.

As fights that end in the eviction of the defender result in an exchange of shells, there is the suggestion that such encounters are to the mutual benefit of the crabs, and may be a process of 'negotiation' rather than aggression (Hazlett 1978; Hazlett 1987; Hazlett 1996). Here, it is possible that the defender also experiences a gain in shell quality if it were at first occupying a shell that was too large and therefore costly to carry. Here, the non-initiator must gain information about the potential shell quality of the initiator, and it is suggested that the function of shell rapping is to advertise this via the fundamental frequency of the individual raps (Hazlett 1987). However, this possibility has been much refuted (Elwood & Glass 1981) as the vigour and pattern of shell-rapping has been shown to be a key determinant of the outcome of hermit crab shell fights (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998; Elwood & Neil 1992). Shell rapping is thus assumed to act as an honest signal (Briffa 2006) of attacker quality as it is costly to perform (Briffa & Elwood 2001).

Like the crustaceans previously mentioned, hermit crabs have been shown to use deceptive display behaviours (bluffs) prior to shell fighting (Elwood et al. 2006). However, these signals are conventional, and thus are open to invasion by cheats. The shell-rapping system used by hermit crabs is a genuinely costly signal, and is therefore presumably immune to invasion by bluffing individuals. This is supported by investigation of physiological costs and the analysis of signal residuals (Briffa 2006) from shell rapping. Therefore hermit crabs may be able to bluff their pre-fight displays (but see Laidre & Elwood 2008), but ultimately fighting ability will be demonstrated during shell-rapping. In attackers, persistence appears to be associated with reaching a physiological threshold of high lactate levels. This is not the case in defenders, but there are clear energetic differences between winners and losers.

Defenders that successfully resist the attacker appear to mobilise their energy stores to a greater extent than those that give up (Briffa & Elwood 2004). Thus, physiological limitations to behaviour may be imposed as metabolic costs incurred through the performance of demanding activity. However, the physiological status of an animal may also dictate its behaviour, predisposing it to perform certain behaviours, or at particular levels, during a contest.

### ***Thesis aims***

In this thesis I present a series of experiments that investigate proximate physiological and whole-body correlates of contest behaviour, using hermit crab shell fights as a model system. The aims are to provide insight into the mechanisms that govern contest behaviour, and to investigate components that may help our understanding of agonistic signalling. For example, many previous studies have analysed the energetic and endocrinological consequences of contest behaviour, which are now well-understood. However, other mechanisms that may affect fighting ability remain poorly investigated. The questions addressed in each chapter are outlined below:

**Chapter 2:** The first experimental chapter investigates the effects of circulating metal ions on the fighting ability of hermit crabs. The ions investigated were calcium ( $\text{Ca}^{2+}$ ) and magnesium ( $\text{Mg}^{2+}$ ). Both of these ions are known to increase the oxygen affinity of haemocyanin, the crustacean respiratory pigment (Nies et al. 1992; Terwilliger 1998; Truchot & Lallier 1992). Thus, it was predicted that higher concentrations of these ions would increase the fighting ability of the crabs by enhancing their aerobic capacity. However, as  $\text{Mg}^{2+}$  can have a narcotising effect on muscle function it was also predicted that there may be a trade-off in its regulation such that the scope for activity may be slightly reduced while aerobic capacity is increased, thereby delaying the consequences of anaerobic respiration.

**Chapter 3:** In this chapter, the effects of the concentration of circulating haemocyanin ( $[\text{Hc}]_{\text{HL}}$ ) on the vigour and outcomes of hermit crab shell fights were analysed. As higher  $[\text{Hc}]_{\text{HL}}$  would lead to higher aerobic capacities, it was predicted that successful contestants would have higher  $[\text{Hc}]_{\text{HL}}$ , allowing them a greater scope for activity before the onset of anaerobic respiration.

**Chapter 4:** As repeated signals are often assumed to advertise the stamina of the sender, this chapter investigated the effects of stamina on fighting ability using ‘performance capacities’ as a measurement of stamina. It was predicted that individuals with higher levels of stamina would be more likely to win shell fights as they could rap more vigorously in the attacker role, or persist inside the shell for longer in the defender role.

**Chapter 5:** In this chapter, the quality of the abdominal muscles of the crabs was investigated in relation to fighting ability. As the previous chapter focussed on stamina, here the muscle protein concentration and muscle/body weight ratios were used as a proxy for strength. It was predicted that both attackers and defenders with better quality muscles (i.e. higher concentrations of muscle protein and higher muscle/body weight ratios) would be better competitors in shell fights as: i) attackers would be able to perform more powerful raps, which increase their chances of winning (Briffa & Elwood 2002) and ii) defenders would be able to hold onto the contested shell more strongly, requiring a greater force to evict them.

**Chapter 6:** As Chapter 4 had investigated whole-organism performance, Chapter 6 again investigated the whole organism, but with a more cognitive approach. The aims of this chapter were to identify whether stable behavioural syndromes, a component of ‘animal personalities’ existed in *P. bernhardus* and whether these were correlated with fighting ability or ‘state’ in terms of aerobic capacity, measured as  $[Hc]_{HL}$ . As this species has been previously demonstrated to possess ‘personality’ (Briffa et al. 2008b), it was likely that behavioural syndromes might be identified. It was predicted that any syndromes identified might influence contest behaviour by altering an individual’s motivation and propensity to engage the opponent (see Reaney & Backwell 2007). However, it was also predicted that the physiological ‘state’ of an individual in terms of its aerobic capacity would be more important in determining fighting ability by limiting the intensity of behaviour that each competitor was capable of performing.

Thus, the correlates of contest behaviour covered by this thesis range from simple ions through more complex structures including proteins, to muscle groups, then physical whole animal measures and finally to abstract whole animal behavioural measures termed ‘personality’.

## CHAPTER 2

### **The role of circulating metal ions during shell fights in the hermit crab *Pagurus bernhardus*.**

Published as:

Mowles, S. L., Briffa, M., Cotton, P. A. & Spicer, J. I. 2008: The role of circulating metal ions during shell fights in the hermit crab *Pagurus bernhardus*. *Ethology* 114, 1014-1022.

### ***Abstract***

Fighting animals must make a series of decisions, and understanding the proximate causes behind these decisions can give insight into how they are made. For example, fights have been analysed with respect to energetic costs and endocrine changes associated with engaging. However, another mechanism for the control of demanding activity, such as fighting, is the modulation of synaptic and muscular function by divalent metal ions. This study examined post-fight haemolymph levels of magnesium ( $Mg^{2+}$ ) and calcium ( $Ca^{2+}$ ) ions in the common European hermit crab *Pagurus bernhardus*. Hermit crabs fight over the ownership of gastropod shells, where they adopt two very different roles during the encounter: attacker and defender. Despite the two roles performing different activities, it was found that  $Mg^{2+}$  and  $Ca^{2+}$  affected them similarly, with concentrations of these ions being higher in successful individuals. Haemolymph concentrations of  $Mg^{2+}$  and  $Ca^{2+}$  were also found to increase as a result of fighting, and these elevated levels may, via allosteric interactions, increase the oxygen affinity of the respiratory pigment haemocyanin, enhancing respiratory capacity and therefore fighting ability. Furthermore, the present study revealed that seasonal changes in circulating levels, along with the ability of competitors to respond to them, may ultimately influence an individual's success in aggressive interactions.



## INTRODUCTION

Contests in animals involve a series of decisions, where opponents must first decide whether to engage in an encounter, then whether to escalate, and finally whether to terminate it by deciding to withdraw or 'give up'. This 'giving up' decision, made by the loser, is of key importance as it determines both the duration and the outcome of the contest. Theoretical studies (e.g. Enquist & Leimar 1983; Enquist et al. 1990; Hammerstein & Parker 1982) assume that decision rules may be based on information about: (a) the perceived value of the resource (RV, for a review see Arnott & Elwood 2008), and (b) the disparity in fighting ability between opponents (relative RHP). Such mutual assessment is widespread in nature (e.g. Burmeister et al. 2002; Clutton-Brock et al. 1979; Thornhill 1984), and usually involves assessment of cues that reflect opponent quality such as body size (e.g. Caldwell & Dingle 1979; Riechert 1978), weapon size (e.g. Barki et al. 1997; Sneddon et al. 1997) or ornamentation (e.g. Lailvaux et al. 2005; Vanhooydonck et al. 2005). However, information about the opponent may not always be available, and is unlikely to be the sole basis for the 'giving up' decision (e.g. Payne & Pagel 1996; Payne & Pagel 1997; Taylor & Elwood 2003).

Agonistic behaviour, including signalling, can be energetically demanding to perform (e.g. Briffa & Elwood 2001; Briffa & Elwood 2004; DeCarvalho et al. 2004; Hack 1997). Thus, the use of agonistic signals may be directly constrained by the ability to meet the immediate costs (*sensu* Hurd 1997) of performance, and 'repeated signals' (Payne & Pagel 1997) are assumed to reveal 'stamina', a key aspect of sender RHP. There is therefore the potential to base strategic decisions on an assessment of the opponent's performance, as well as on the cost incurred by engaging in the contests. In order to test the idea that strategic decisions are influenced by performance costs, fights have increasingly been analysed with respect to the proximate physiological consequences of agonistic behaviour, including energetic costs (Briffa & Elwood 2001; Neat et al. 1998; Schuett & Grober 2000; Sneddon et al. 1999; Thorpe et al. 1995) and endocrine changes (Briffa & Elwood 2007; Sneddon et al. 2000b) across a range of taxa. In crustaceans, high lactate, which appears to accumulate during bouts of demanding fight activities, appears to be a key proximate mechanism that influences fight outcomes (Briffa & Elwood 2001; Briffa & Elwood 2005; Sneddon et al. 1999).

Systems that cope with elevated lactate may play a role in extending persistence in a fight and thus delaying the decision to withdraw. The circulating metal ions  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  have

the ability to modulate respiratory performance. Dissolution of the  $\text{CaCO}_3$  exoskeleton can be used by crustaceans to buffer against acid-base balance disturbances, including those caused by elevated lactate, resulting in an increase in  $[\text{Ca}^{2+}]_{\text{HL}}$  (Cameron 1985; DeFur et al. 1980; Henry et al. 1981). This is not restricted to the Crustacea however, and an analogous system is also present in vertebrates with a carapace, such as the painted turtle *Chrysemys picta* (Jackson 2000). Furthermore, both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  increase haemocyanin- $\text{O}_2$  affinity, thus potentially improving  $\text{O}_2$  transport under conditions of low  $\text{O}_2$  availability, either due to functional (strenuous activity) or environmental hypoxia (Mangum 1997; Truchot 1975). Ionic regulation is thus a critical process, and is of particular importance in crustaceans (Robertson 1949), with the concentration of  $\text{Mg}^{2+}$  affecting both overall levels of aggression (Holliday 1980), and activity (for a review see Morritt & Spicer 1993) across taxa (Walters & Uglow 1981).

In crustaceans, however, the benefits of these ions will be associated with a cost.  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  continually diffuse from the external sea water environment into the haemolymph, and  $\text{Mg}^{2+}$  is actively excreted again (Cornell 1979). This is in order to avoid a narcotising effect acting at the neuromuscular synapse (Boardman & Collier 1946; Purves et al. 2001) and directly on muscle tissue (Fabiato & Fabiato 1975; Sigel & Sigel 1990). Indeed,  $\text{Mg}^{2+}$  is actively regulated such that levels are maintained at a lower concentration than that of sea water (Robertson 1949). This active process requires energy, and crustaceans appear to be better able to regulate both  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  at higher temperatures. As a consequence,  $\text{Mg}^{2+}$  levels are also known to vary seasonally (McAllen et al. 2005; Spicer et al. 1994), and with latitude (Frederich et al. 2000a; Frederich et al. 2000b; Frederich et al. 2001). Regardless of season, any beneficial effect of up-regulating levels of circulating metal ions in terms of  $\text{O}_2$  transport, which in the context of aggressive encounters may allow the decision to withdraw to be delayed, will come at the cost of a potential decrease in the efficiency of muscle contraction. Nevertheless, it is clear that crustaceans are able to modulate levels of circulating metal ions and that circulating levels affect activity including aggression (Holliday 1980). The aim of this study was to determine whether they also play a role in strategic decision making during agonistic encounters.

Hermit crabs (Decapoda: Anomura) fight over the ownership of gastropod shells, with the opponents adopting two very different roles during the encounter. The initiating crab or 'attacker' attempts to take the shell of the non-initiator or 'defender'. The attacker, having first overpowered the defender, grips the shell of the defender and proceeds to perform bouts of 'shell rapping' by bringing its own shell rapidly and repeatedly into contact with that of the

defender. A series of such bouts, separated by distinct pauses, are performed during the shell fight. The vigour of rapping, and therefore presumably the advertised fighting ability of the attacker (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998), varies in terms of the number of raps in each bout, the duration of the pauses between bouts (Briffa et al. 1998), and the force of the raps (Briffa & Elwood 2002). Shell fights can end in one of two ways, either the defender decides to give up; allowing itself to be evicted by the attacker, which removes the defender through the aperture of the shell; or the attacker may decide to give up first without having evicted the defender. The vigour of shell-rapping has been shown to be a key determinant of the outcome of hermit crab shell fights (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998), acting as an honest signal (Briffa 2006) of attacker quality as rapping is costly to perform (Briffa & Elwood 2001; Briffa & Elwood 2004).

The aim of this study was to determine how circulating levels of magnesium and calcium in the haemolymph ( $[Mg^{2+}]_{HL}$  and  $[Ca^{2+}]_{HL}$  respectively) are related to strategic decisions and the vigour of agonistic behaviour, using hermit crab shell fights as a model system. As the haemolymph concentration of both of these metal ions has been shown to vary seasonally (McAllen et al. 2005; Spicer et al. 1994), a further aim was to determine whether such changes could drive seasonal variation in fight dynamics.

## MATERIALS AND METHODS

### *Study organisms*

Hermit crabs (*Pagurus bernhardus*) were collected from rock pools at Hannaford Point, Cornwall, UK (50° 20' N, 4° 27' W), during two separate periods: between February and April ('spring'), then again between June and July ('summer') 2006 to give two clear seasonal treatments. During the autumn and winter the reproductive behaviour of crabs influences aggression and shell preferences (Elwood & Neil 1992; Neil & Elwood 1985). For this experiment, where the aim was to compare aggressive behaviour between seasons, we therefore avoided these potential changes in motivation by not conducting fights during these reproductive time periods. The hermit crabs were kept in groups of 80-150 in plastic aquaria (vol. ~50 l, dimensions 65cm x 50cm x 30cm), filled with aerated sea water to a depth of 15cm. These were maintained at 15°C in a temperature controlled environment on a 12/12h light/dark cycle. The crabs were fed *ad libitum* on a diet of catfish pellets and were returned to the shore within a fortnight of collection.

Hermit crabs were removed from their gastropod shells by carefully cracking the shells open in a bench vice. They were then sexed and examined for physical damage. Only intermoult male crabs, free from obvious parasites and physical damage, were used in the experiment in order to avoid sex-related behavioural differences as found in previous studies (Neil & Elwood 1985). Unused crabs were provided with new shells and were returned within two weeks to the shore from which they were originally collected.

The study crabs were assigned to pairs consisting of a small crab and a large crab (mean weights  $\pm$  SE.; small,  $0.735 \pm 0.013$ g; large,  $1.009 \pm 0.021$ g). The larger crab of the pair (potential attacker) was provided with a *Littorina littorea* shell that was only 50% of its preferred shell weight, while the smaller crab (potential defender) was provided with a *L. littorea* shell which would be 100% adequate for the larger crab of the pair. The preferred shell size was determined using a regression which related crab weight to preferred shell weight (Briffa & Elwood 2007).

Following shell allocation, crabs were isolated in crystallizing dishes (diam. = 95mm) filled with aerated sea water at 15°C, and allowed to acclimate for 16 hours prior to being used in staged fights. Encounters were staged in two groups to which the pairs of crabs were randomly allocated. In the control group ( $N = 12$  pairs: 6 'spring', 6 'summer'), haemolymph samples were taken from crabs when the fight was terminated at the point where the attacker

made initial contact with the shell of the defender. This ensured that control samples were taken from crabs which were prepared to fight but did not fight. The second group ( $N = 50$  pairs: 29 'spring', 21 'summer') comprised fights that were allowed to proceed until their conclusion, where either the defender decided to allow itself to be evicted from its shell ( $N = 38$  pairs: 23 'spring', 15 'summer'); or the attacker decided to terminate the fight without first having evicted the defender ( $N = 12$  pairs: 6 'spring', 6 'summer').

### ***Behavioural Observations***

Fights were staged between 0830 and 1200 hours each day. A large crab was placed in an arena consisting of a crystallizing dish (diam. = 95mm) with a 1cm thick sandstone tile as a base, and filled to a depth of 4cm with aerated seawater. The dish was then placed in an observational chamber, behind a two-way mirror, so that the crabs could not see the observer. The small crab was then introduced into the arena containing the large crab and the ensuing behaviours were recorded for both crabs using a Psion Workabout MX (Psion Teklogix Inc., Mississauga, ON, Canada) configured as a time-event recorder using The Observer 5.0 (Noldus IT, Wageningen, The Netherlands) event-recording software. The parameters of the pattern of shell rapping (see Figure 2.1) were then analysed in order to calculate the attacker vigour for each fight.

### ***Assay of Magnesium and Calcium***

Immediately after the fight, the crabs were removed from their shells, and haemolymph samples (approx. 30 $\mu$ l) were removed from each crab using a syringe (BD Micro-Fine™ 1mL, Becton, Dickinson and Company, NJ, USA), the needle of which (29 G) was inserted into the infrabranchial sinus, *via* the arthroal membrane at the base of the third pereopod. Haemolymph samples were placed on ice within 90 sec of the end of the fight, and were then stored at -20°C. For analysis, the haemolymph samples were defrosted and diluted serially with ultra pure water (Elga Maxima, High Wycombe, UK) in order to bring the concentration of the metal ion under investigation within the optimum working range of the Varian Flame Atomic Absorbance Spectrophotometer (F.A.A.S). The concentrations of Mg<sup>2+</sup> and Ca<sup>2+</sup> were then determined with flame atomic absorbance spectroscopy using a nitrous oxide-acetylene flame (Athanasopoulos 1994; U.S.E.P.A. 1983).

The haemolymph samples were diluted by a factor of 351x and analysed at a  $\lambda$  of 422.7nm in order to determine  $[Ca^{2+}]_{HL}$  at the optimum working range of the F.A.A.S. (1-4 $\mu$ g/mL). In order to determine  $[Mg^{2+}]_{HL}$ , haemolymph used to make the  $Ca^{2+}$  samples was further diluted to a factor of 3071.25x in order to meet the optimum working range for magnesium in the instrument (0.1-0.4 $\mu$ g/mL), and analysed at a  $\lambda$  of 285.2nm.

BEHAVIOURS RECORDED		
<i>Name</i>	<i>Type</i>	<i>Description</i>
'Rap'	Event	Attacker performs a single shell rap.
'Contact'	State	Attacker crab contacts the defender's shell with its chelipeds.
'Insert chelipeds'	State	Attacker inserts its chelipeds into the aperture of the defender's.
'Other'	State	The crab performs another behaviour with its chelipeds (default behaviour).
'Eviction'	Event	Attacker evicts the defender from its shell.
'Grapple'	State	Mutual grapple.
CALCULATED INDICES OF ATTACKER VIGOUR		
'Contest duration'		The duration of the contest in seconds, as timed from the first shell rap until the last.
'Total raps'		The total number of shell raps during the contest.
'Total bouts'		The total number of bouts of shell rapping during the contest.
'Raps/Bout'		The total number of shell raps divided by the total number of bouts, giving the mean number of raps per bout.
'Mean pause'		The mean duration of pauses between bouts of shell rapping,

**Figure 2.1.** Ethogram of shell rapping behaviours recorded and calculated indices of attacker vigour.

RESULTS

EFFECT OF FIGHT OUTCOMES

In order to determine the effects of fighting, outcome, and season on haemolymph metal ion concentrations in hermit crabs, repeated measures ANOVAs were performed as an attacker and defender are subjects within each fight. The factors included in the models were ‘outcome’ (eviction or non-eviction), and ‘season’ (spring or summer), the repeated measure was ‘role’ (attacker or defender) and the dependent variable was either magnesium or calcium concentration.

Magnesium

Repeated Measures ANOVA – Magnesium in all individuals

Overall, there was no difference in the  $[Mg^{2+}]_{HL}$  between the fight outcomes ( $F_{2,55} = 0.131$ ,  $P = 0.8773$ ; Figure 2.2). However,  $[Mg^{2+}]_{HL}$  differed between the roles ( $F_{1,55} = 5.505$ ,  $P = 0.0226$ ; Figure 2.3), being higher in defenders than attackers.

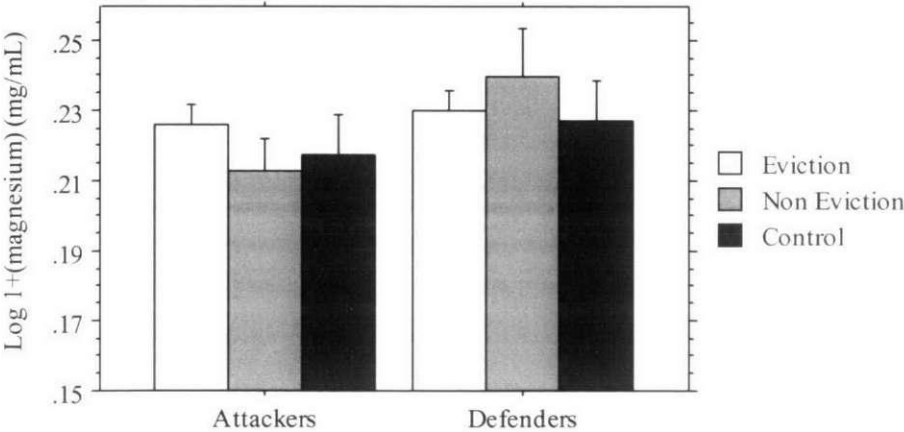
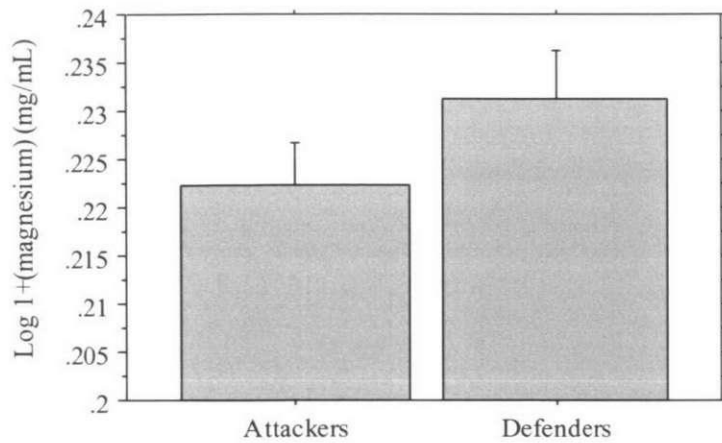
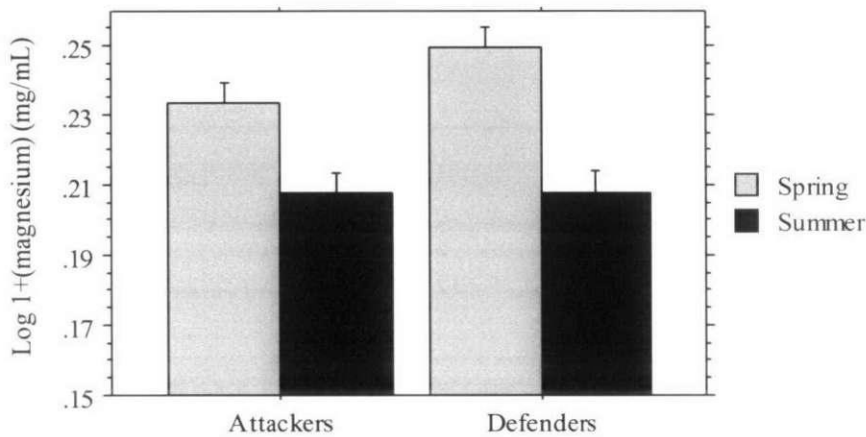


Figure 2.2.  $[Mg^{2+}]_{HL}$  in each role in relation to the outcome of the fight.



**Figure 2.3.**  $[Mg^{2+}]_{HL}$  in each role post-fight.

The  $[Mg^{2+}]_{HL}$  was also found to differ between the seasons, being higher in the spring than in the summer ( $F_{1,55} = 15.862$ ,  $P = 0.0002$ ; Figure 2.4).

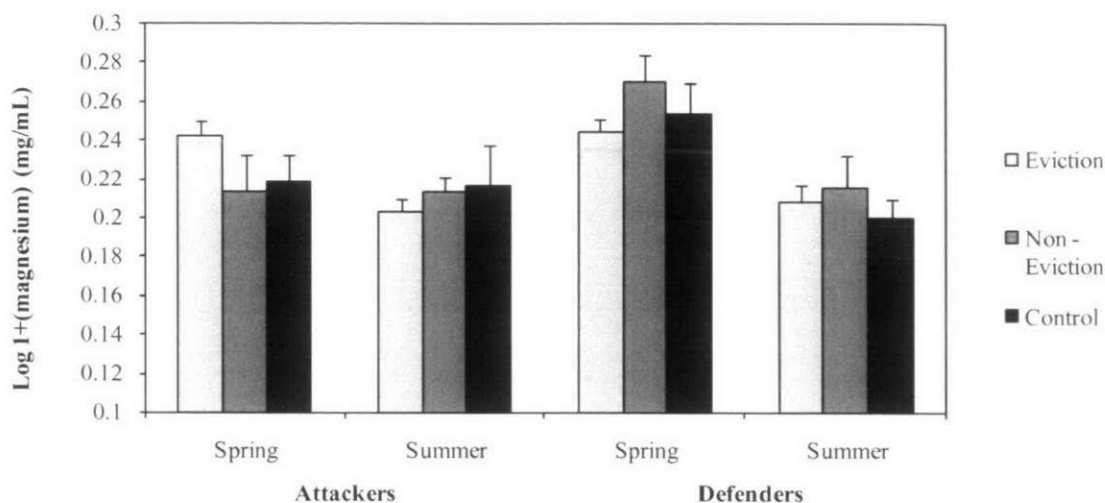


**Figure 2.4.**  $[Mg^{2+}]_{HL}$  in each role in relation to the season in which the fight was performed.

There was also a significant interaction effect between the role adopted by the crab during the fight and the season in which the fight was performed ( $F_{1,55} = 7.857$ ,  $P = 0.0070$ ; Figure 2.4), indicating that the seasonal change in  $[Mg^{2+}]_{HL}$  is more marked in defenders than in attackers.

A three-way interaction was also detected between the role adopted by the crab, the season in which the fight was performed, and the outcome of the fight ( $F_{2,55} = 3.260$ ,  $P = 0.0459$ ; Figure 2.5).



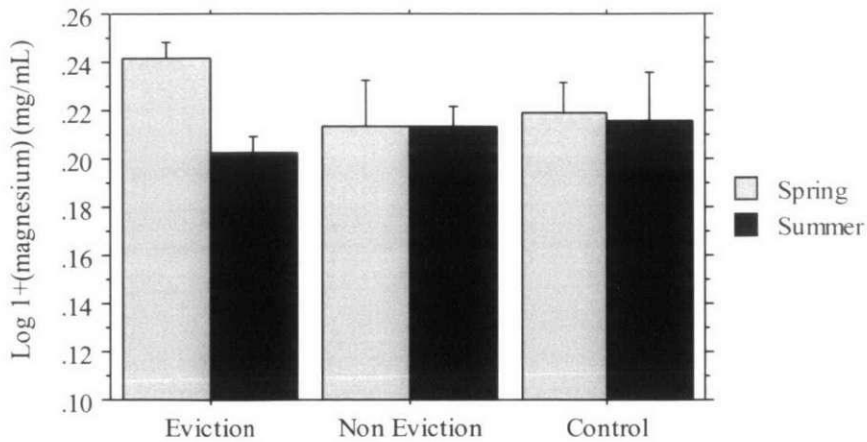


**Figure 2.5.** [Mg<sup>2+</sup>]<sub>HL</sub> in each role in interaction with the outcome of the fight, and the season in which the fight was performed.

### *ANCOVA – Magnesium in Attackers only*

Interaction effects may mask differences within each role. In order to examine this possibility, two-factor ANCOVAs were used to examine each role independently. The [Mg<sup>2+</sup>]<sub>HL</sub> was the dependent variable, while the weight of the crabs was the covariate. The factors were ‘outcome’ (eviction or non-eviction), and ‘season’ (spring or summer).

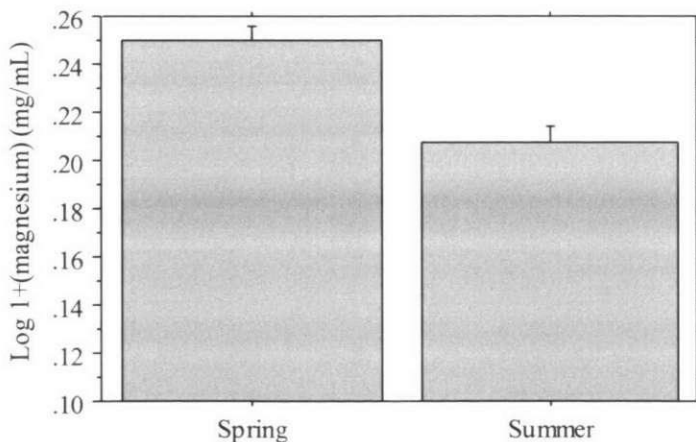
When attackers were analysed on their own, there was no three-way interaction involving the covariate and the two factors. This was thus deleted and the model recalculated. There was no effect of outcome ( $F_{2,51} = 1.894$ ,  $P = 0.1610$ ), season ( $F_{1,51} = 0.031$ ,  $P = 0.8612$ ) or weight ( $F_{1,51} = 2.383$ ,  $P = 0.1289$ ). There were no significant interactions between attacker weight and outcome ( $F_{2,51} = 1.170$ ,  $P = 0.1910$ ) or season ( $F_{1,51} = 0.035$ ,  $P = 0.8518$ ). However, a significant interaction between outcome and season ( $F_{2,51} = 3.834$ ,  $P = 0.0281$ ; Figure 2.6) indicates that the [Mg<sup>2+</sup>]<sub>HL</sub> in attackers increases as a result of fighting in the spring, whereas fighting in the summer appears to have no effect.



**Figure 2.6.**  $[Mg^{2+}]_{HL}$  in attackers in relation to the outcome, and the season in which the fight was performed.

### *ANCOVA – Magnesium in Defenders only*

Again, in order to investigate possible interaction effects, defenders were analysed independently of attackers. The concentration of metal ions was the dependent variable in these models, while the weight of the crabs was the covariate. The factors were fight ‘outcome’ (eviction or non-eviction) and ‘season’ (spring or summer). In defenders there were no significant interaction effects and these were deleted from the model. No significant difference was found in the  $[Mg^{2+}]_{HL}$  between fight outcomes ( $F_{2,56} = 0.896$ ,  $P = 0.4141$ ) and there was no significant effect of defender weight on  $[Mg^{2+}]_{HL}$  ( $F_{1,56} = 0.170$ ,  $P = 0.6821$ ). However,  $[Mg^{2+}]_{HL}$  was significantly higher in the spring than in the summer ( $F_{1,56} = 24.808$ ,  $P < 0.0001$ ; Figure 2.7).



**Figure 2.7.**  $[Mg^{2+}]_{HL}$  in defenders in relation to the season in which the fight was performed.

Calcium

Repeated Measures ANOVA – Calcium in all individuals

Similarly to the analysis of magnesium, a repeated measures ANOVA was performed to determine the effects of outcome and season on  $[Ca^{2+}]_{HL}$ . The factors included in the model were ‘outcome’ (eviction or non-eviction), and ‘season’ (spring or summer), while the repeated measure was ‘role’ (attacker or defender).

There was no difference in  $[Ca^{2+}]_{HL}$  between the fight outcomes ( $F_{2,54} = 0.675$ ,  $P = 0.5133$ ; Figure 2.8). However, a significant interaction effect between role and outcome ( $F_{2,54} = 5.723$ ,  $P = 0.0056$ ; Figure 2.8) indicates that  $[Ca^{2+}]_{HL}$  is higher in attackers that effected an eviction than in those that gave up or did not fight, whereas  $[Ca^{2+}]_{HL}$  was higher in non-evicted defenders than in those that were evicted.

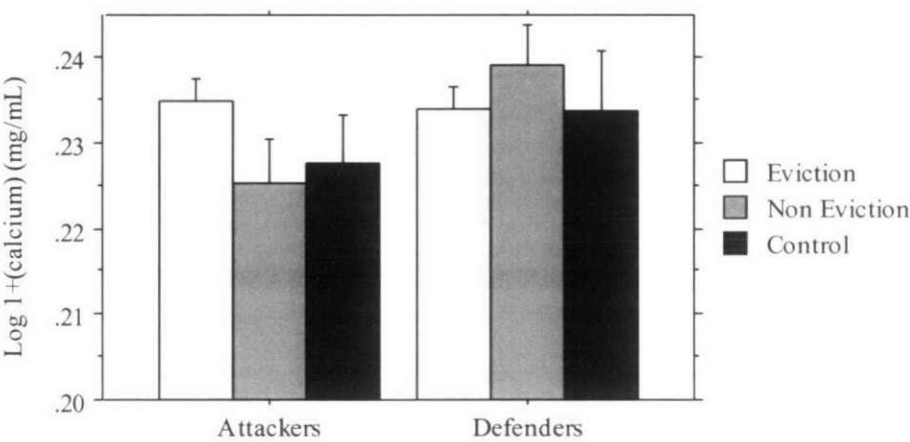
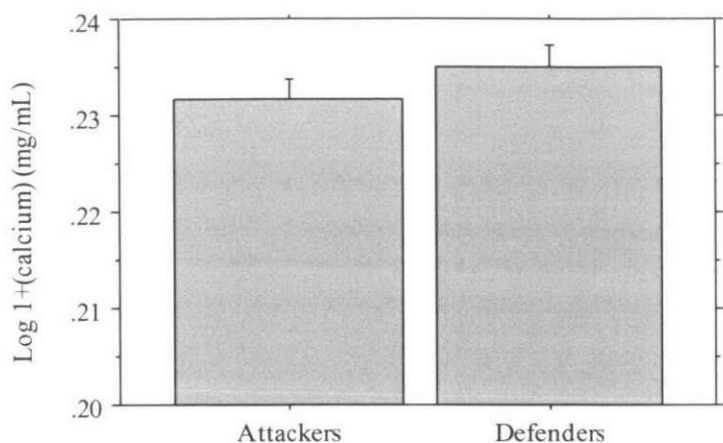


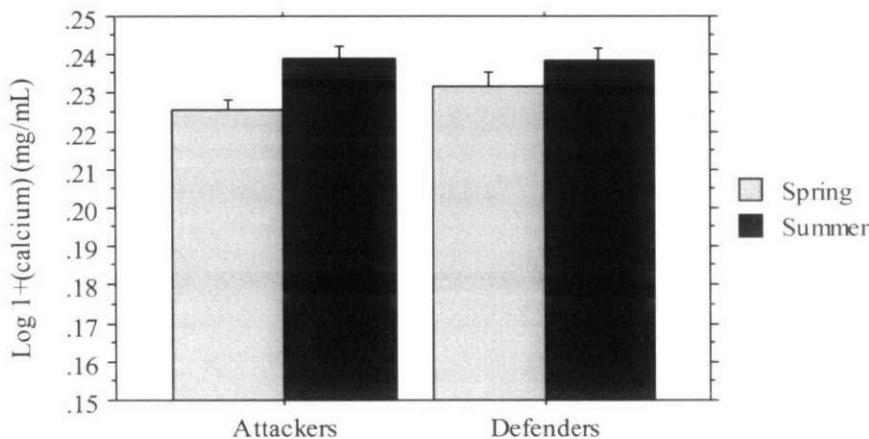
Figure 2.8.  $[Ca^{2+}]_{HL}$  in each role in relation to the outcome of the fight.

The  $[Ca^{2+}]_{HL}$  was also significantly different between the two roles, defenders having higher  $[Ca^{2+}]_{HL}$  than attackers ( $F_{1,54} = 9.576$ ,  $P = 0.0031$ ; Figure 2.9).



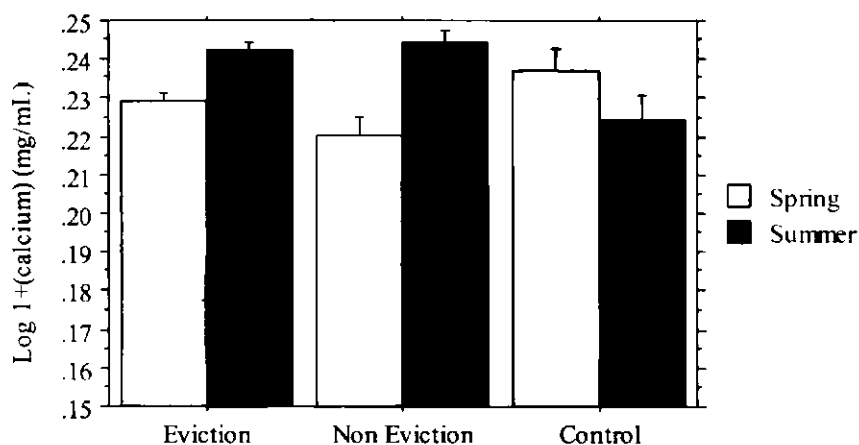
**Figure 2.9.**  $[Ca^{2+}]_{HL}$  in each role post-fight.

The  $[Ca^{2+}]_{HL}$  was found to be significantly higher in the summer than in the spring ( $F_{1,54}=4.180$ ,  $P=0.0458$ ; Figure 2.10). There was a significant interaction between the role adopted by the crab and season ( $F_{1,54}=7.588$ ,  $P=0.0080$ ; Figure 2.10), indicating that the seasonal change in  $[Ca^{2+}]_{HL}$  is more marked in attackers than in defenders.



**Figure 2.10.**  $[Ca^{2+}]_{HL}$  in each role in relation to the season in which the fight was performed.

There was also a significant interaction effect between the season in which the fight occurred, and the outcome of the fight ( $F_{2,54}=5.764$ ,  $P=0.0054$ ; Figure 2.11). In spring,  $[Ca^{2+}]_{HL}$  is higher in control crabs that did not fight than in crabs that fought whereas in summer  $[Ca^{2+}]_{HL}$  is lower in control crabs than in crabs that fought.

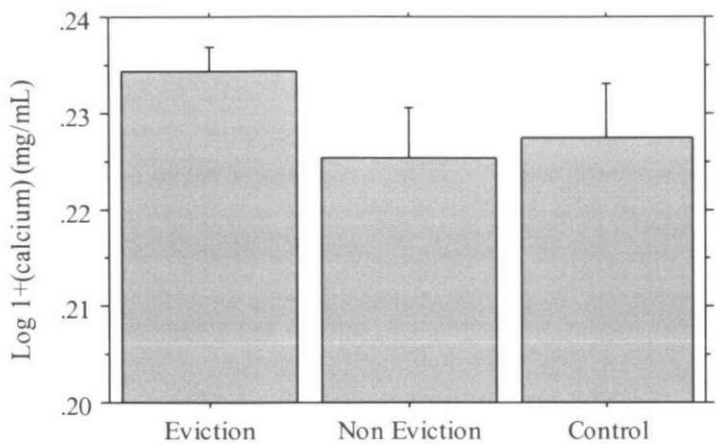


**Figure 2.11.**  $[Ca^{2+}]_{HL}$  in relation to the outcome, and to the season in which the fight was performed.

### *ANCOVA – Calcium in Attackers only*

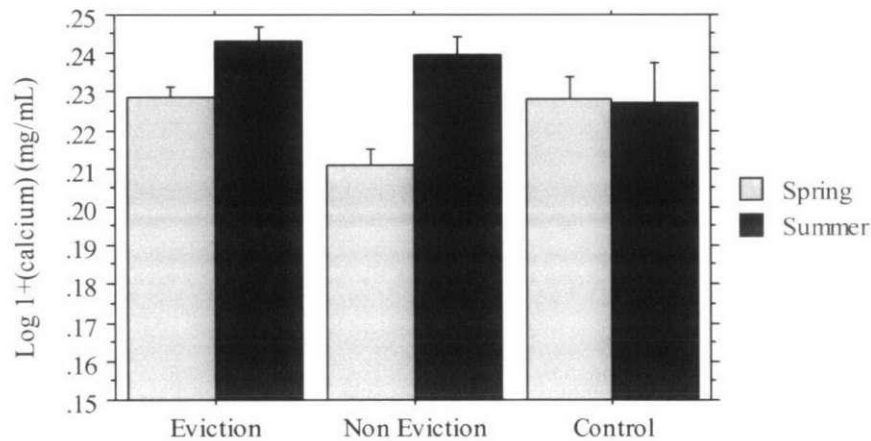
As with the magnesium analysis, interaction effects may mask differences between the roles. Two-factor ANCOVAs were therefore used to examine the effects of calcium on each role independently.

When attackers were analysed on their own, there was no three-way interaction involving the covariate and the two factors. This was thus deleted and the model recalculated. There was a non-significant trend for outcome ( $F_{2,52} = 2.545$ ,  $P = 0.0882$ ; Figure 2.12) where attackers that effected an eviction appear to have higher  $[Ca^{2+}]_{HL}$  than both controls, and attackers that failed to evict the defender. However, further analysis revealed that the apparent difference between outcomes was a non-significant trend for higher haemolymph calcium in successful attackers than unsuccessful ones ( $F_{1,43} = 3.173$ ,  $P = 0.0819$ ), while there was no difference between attackers that effected an eviction and controls ( $F_{1,43} = 1.147$ ,  $P = 0.2902$ ).



**Figure 2.12.**  $[Ca^{2+}]_{HL}$  in attackers in relation to the fight outcome.

There was no significant difference in  $[Ca^{2+}]_{HL}$  between the seasons in attackers ( $F_{1,52} = 0.781$ ,  $P = 0.3810$ ), and no effect of weight ( $F_{1,52} = 0.374$ ,  $P = 0.5433$ ). There were no significant interactions between attacker weight and outcome ( $F_{2,52} = 2.222$ ,  $P = 0.1185$ ) or season ( $F_{1,52} = 0.194$ ,  $P = 0.6618$ ). However, a two-way interaction was found between outcome and season ( $F_{2,52} = 5.187$ ,  $P = 0.0088$ ; Figure 2.13). In spring, unsuccessful attackers had lower  $[Ca^{2+}]_{HL}$  compared to attackers that evicted the defender or did not fight, in summer, however, this difference between unsuccessful attackers and those that evicted the defender or did not fight was less marked.

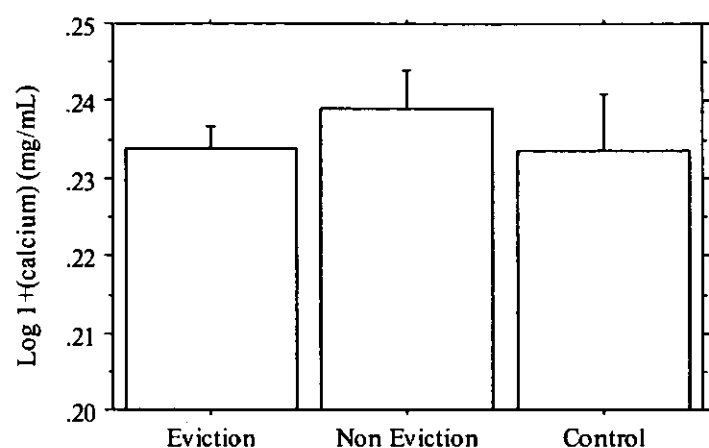


**Figure 2.13.**  $[Ca^{2+}]_{HL}$  in attackers in relation to the outcome, and to the season in which the fight was performed.

*ANCOVA – Calcium in Defenders only*

When defenders were analysed on their own there were no significant interactions involving

the covariate, and these were thus deleted from the model, which was recalculated. There was no effect of outcome ( $F_{2,55} = 0.373$ ,  $P = 0.6903$ ; Figure 2.14), season ( $F_{1,55} = 1.846$ ,  $P = 0.1798$ ) or weight ( $F_{1,55} = 0.033$ ,  $P = 0.8559$ ) on  $[Ca^{2+}]_{HL}$ .



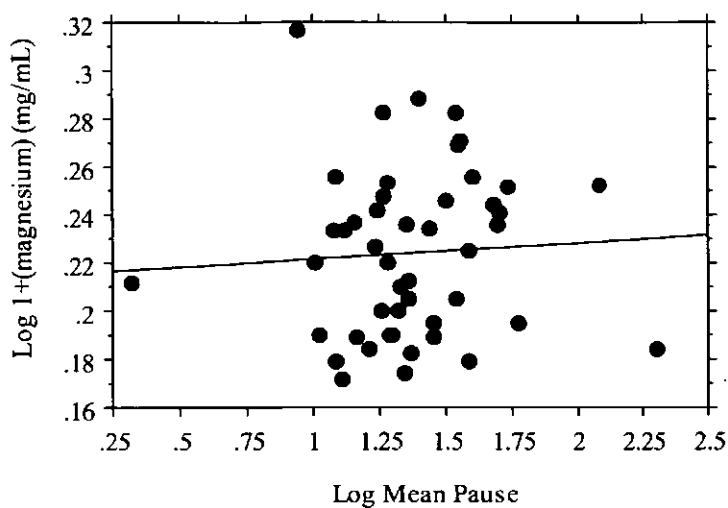
**Figure 2.14.**  $[Ca^{2+}]_{HL}$  in defenders in relation to the fight outcome.

### **RELATIONSHIPS BETWEEN FIGHT VIGOUR AND METAL ION CONCENTRATIONS**

In order to determine the effects of vigour on metal ion concentration, a series of two-factor ANCOVAs were performed that included the haemolymph concentration of the metal ion as the dependent variable, and parameters of the pattern of shell rapping (i.e. contest duration in seconds, number of raps per fight, number of bouts of rapping per fight, mean number of raps per bout, and mean pause duration) as covariates. Fight outcome and season were included in the models as these factors have been shown in the previous section to be influenced by the concentration of metal ions present. Bivariate scattergrams with regression lines have been presented in order to illustrate the direction of the effects.

#### ***Magnesium***

No significant effects were found between the  $[Mg^{2+}]_{HL}$  and the parameters of the pattern of shell rapping (see ethogram in Materials and Methods). In attackers there was no significant correlation between  $[Mg^{2+}]_{HL}$  and contest duration ( $F_{1,45} = 1.804$ ,  $P = 0.1860$ ), total raps ( $F_{1,45} = 2.684$ ,  $P = 0.1083$ ), total bouts ( $F_{1,45} = 2.080$ ,  $P = 0.1562$ ), and raps/bout ( $F_{1,45} = 0.210$ ,  $P = 0.6491$ ), although there was a non-significant trend for a positive correlation with mean pause duration ( $F_{1,40} = 3.787$ ,  $P = 0.0587$ ; Figure 2.15).



**Figure 2.15.**  $[Mg^{2+}]_{HL}$  in attackers in relation to mean pause duration.

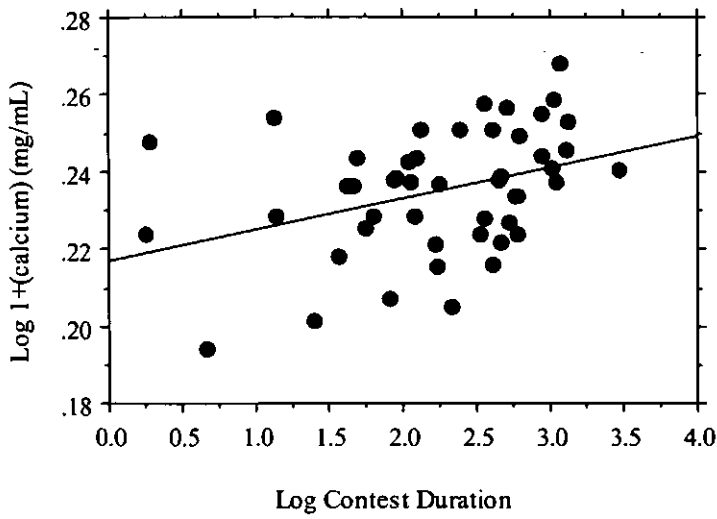
Likewise, in defenders there were no significant relationships between the  $[Mg^{2+}]_{HL}$  and contest duration ( $F_{1,46} = 2.539$ ,  $P = 0.1179$ ), total raps ( $F_{1,46} = 0.788$ ,  $P = 0.3793$ ), total bouts ( $F_{1,46} = 1.350$ ,  $P = 0.2513$ ), raps/bout ( $F_{1,46} = 1.114$ ,  $P = 0.2966$ ), or mean pause duration ( $F_{1,44} = 1.799$ ,  $P = 0.1867$ ).

### **Calcium**

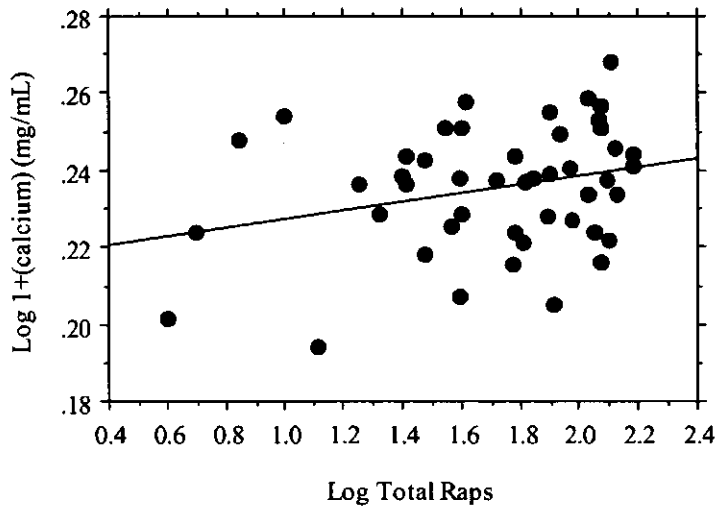
No significant effects were found for attackers between  $[Ca^{2+}]_{HL}$  and the pattern of rapping: contest duration ( $F_{1,46} = 1.716$ ,  $P = 0.1968$ ), total raps ( $F_{1,46} = 1.522$ ,  $P = 0.2235$ ), total bouts ( $F_{1,46} = 1.647$ ,  $P = 0.2058$ ), raps/bout ( $F_{1,46} = 0.056$ ,  $P = 0.8135$ ), and mean pause length ( $F_{1,44} = 2.669$ ,  $P = 0.1095$ ).

However, contrary to the results for attackers, defender  $[Ca^{2+}]_{HL}$  was related to the pattern of shell rapping, in terms of contest duration ( $F_{1,44} = 10.181$ ,  $P = 0.0026$ ; Figure 2.16), as well as the number of raps ( $F_{1,44} = 8.300$ ,  $P = 0.0061$ ; Figure 2.17), and bouts of rapping ( $F_{1,44} = 7.382$ ,  $P = 0.0094$ ) inflicted on them by the attackers.



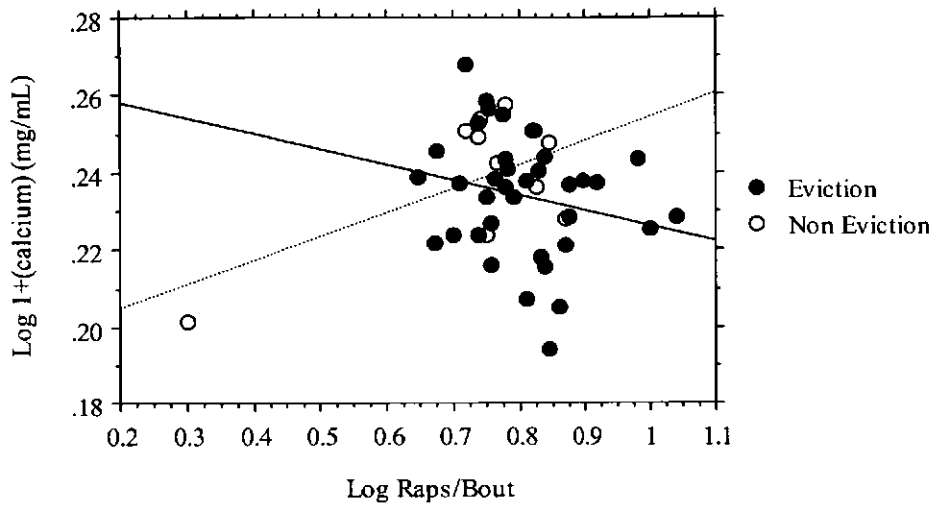


**Figure 2.16.**  $[Ca^{2+}]_{HL}$  in defenders in relation to the duration of the contest.



**Figure 2.17.**  $[Ca^{2+}]_{HL}$  in defenders in relation to the total number of raps in the contest.

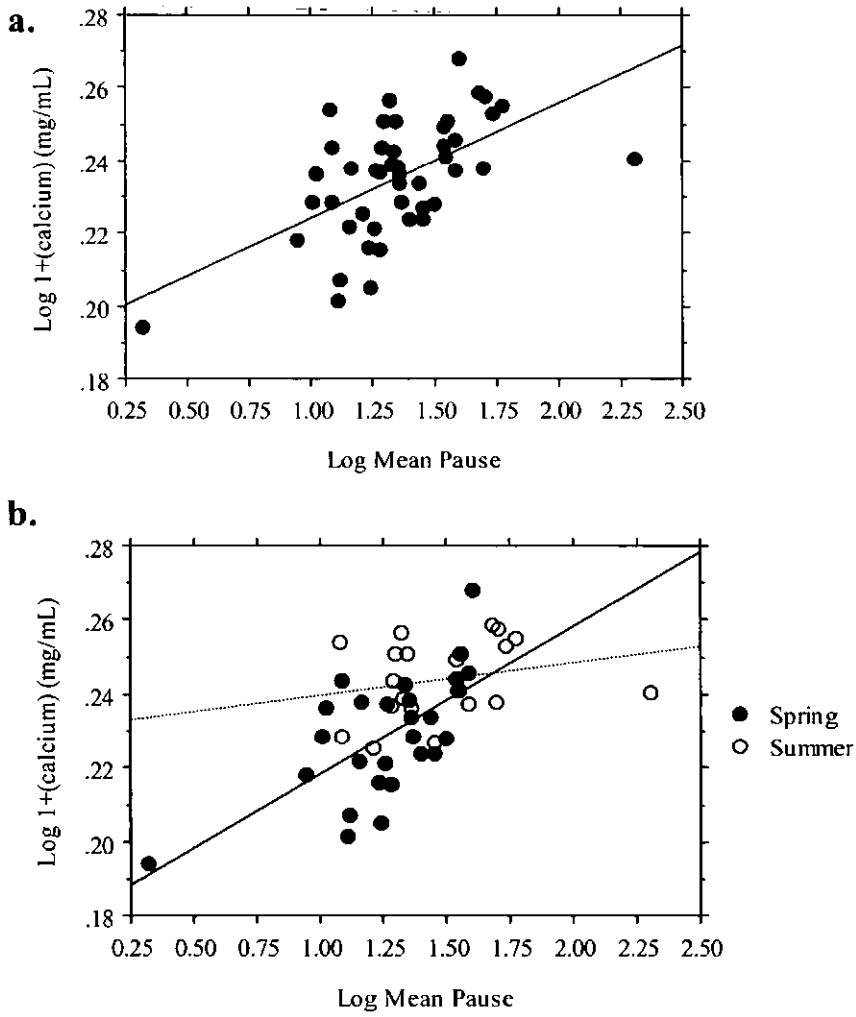
No significant effect was found between the number of raps per bout and  $[Ca^{2+}]_{HL}$  in defenders ( $F_{1,41}=0.058$ ,  $P=0.8103$ ), although a two-way interaction was found between the number of raps per bout and outcome ( $F_{1,41}=4.148$ ,  $P=0.0482$ ; Figure 2.18), where  $[Ca^{2+}]_{HL}$  appears to increase with the number of raps per bout in defenders which retain their shells ( $R^2 = 0.331$ ,  $F_{1,10}=4.956$ ,  $P=0.0502$ ) whereas  $[Ca^{2+}]_{HL}$  appears to decrease with the number of raps per bout in evicted defenders ( $R^2 = 0.051$ ,  $F_{1,34}=1.839$ ,  $P=0.1840$ ).



**Figure 2.18.**  $[Ca^{2+}]_{HL}$  in defenders in relation to the number of raps per bout.

These relationships however, appear to be being driven by an outlier where an attacker performed an unusually low number of raps per bout and a defender had very low  $[Ca^{2+}]_{HL}$ . The removal of this datum point results in the disappearance of the interaction involving the outcome, whilst there is a non-significant negative relationship between the number of raps per bout and defender  $[Ca^{2+}]_{HL}$  ( $F_{1,43} = 3.753$ ,  $P = 0.0593$ ).

A significant effect of mean pause duration was found on  $[Ca^{2+}]_{HL}$  in defenders ( $F_{1,39} = 8.790$ ,  $P = 0.0051$ ; Figure 2.19a), as well as a significant interaction between the mean pause duration and the season in which the fight was performed ( $F_{1,39} = 5.876$ ,  $P = 0.02011$ ; Figure 2.19b). The  $[Ca^{2+}]_{HL}$  appears to increase with the mean pause duration, and this trend is stronger in the spring ( $R^2 = 0.420$ ,  $F_{1,25} = 18.107$ ,  $P = 0.0003$ ) than in the summer ( $R^2 = 0.059$ ,  $F_{1,17} = 1.061$ ,  $P = 0.3174$ ).



**Figure 2.19.**  $[\text{Ca}^{2+}]_{\text{HL}}$  in defenders in relation to mean pause duration.

**a.** Regression line for all defenders, regardless of season.

**b.** Separate regression lines for each season to illustrate the significant interaction between mean pause duration and season.

## DISCUSSION

Levels of circulating metal ions varied significantly during hermit crab shell fights, and were influenced by the role adopted by the crabs (Figures 2.3 and 2.9), engaging in a fight, and the outcome of the fight for those individuals that fought (Figures 2.2, 2.8, 2.12, 2.14).

Circulating levels of metal ions were also found to vary seasonally, although these effects differed between roles, and outcomes (Figures 2.4, 2.5, 2.6, 2.7, 2.10, 2.11, 2.13).

Furthermore, a positive relationship was detected between the  $[Ca^{2+}]_{HL}$  in defenders and the duration of contests (Figures 2.16, 2.17). These results thus indicate that the haemolymph concentrations of metal ions can vary during contests and may also affect the fighting ability of contestants. Defenders were found to have higher  $[Mg^{2+}]_{HL}$  and  $[Ca^{2+}]_{HL}$  than attackers. As defenders are usually smaller than attackers, this could have been a function of size, but as metal ions were not related to the size of the crabs this was not the case, indicating that the differing metal ion levels were due to the different activities performed by the different roles during the fight. Indeed, analysis of unfought control crabs alone revealed that there were no differences in the levels of metal ions between the roles, further supporting the hypothesis that the heightened levels in defenders relative to attackers are a result of changes in concentration that occur during the encounter. It is thus clear that these changes vary with the role adopted by the crab.

It was found that winners had elevated  $[Mg^{2+}]$  and  $[Ca^{2+}]$  compared to crabs that did not fight, although  $[Mg^{2+}]$  and  $[Ca^{2+}]$  did not rise significantly in successful defenders. This indicates that high levels of circulating metal ions are associated with the chance of winning, but particularly in attackers, perhaps by extending the period for which a crab may persist in the encounter. These results are in contrast to a previous study that found no difference in the baseline levels of exoskeleton or haemolymph calcium between winners and losers in American lobster, *Homarus americanus* contests (Vye et al. 1997). We detected differences in the levels of metal ions between winners and losers after taking haemolymph samples immediately after each fight whereas Vye et al. (1997) found no difference in baseline (15h post-fight) calcium levels, further suggesting that the concentrations of ions are regulated in response to engaging in fights.

Hermit crab shell fights are energetically expensive, and the accumulation of *L*-lactate shown in previous studies (Briffa & Elwood 2001; Briffa & Elwood 2004; Briffa & Elwood 2005) indicates that anaerobic respiration is important. Elevated levels of metal ions may reduce the need to rely on anaerobic respiration by increasing the capacity for aerobic

respiration *via* an increase in haemocyanin O<sub>2</sub> affinity (Nies et al. 1992; Terwilliger 1998; Truchot & Lallier 1992). Furthermore, once anaerobic respiration has commenced, Mg<sup>2+</sup> and Ca<sup>2+</sup> may ameliorate the associated costs by buffering against the acidosis caused by the accumulation of *L*-lactate (DeFur et al. 1980; Henry et al. 1981). It is curious, however, that attackers that do not effect an eviction have lower [Ca<sup>2+</sup>]<sub>HL</sub> than both successful attackers and controls (Figure 2.8). The beneficial effects of maintaining high [Ca<sup>2+</sup>]<sub>HL</sub>, coupled with its relatively passive movement into the haemolymph (Cornell 1979) should preclude its decrease during shell fights. Because of this, it is possible that attackers that fail to effect evictions are predisposed to do so as they already have lower than average Ca<sup>2+</sup> prior to engaging in a fight.

While Mg<sup>2+</sup> and Ca<sup>2+</sup> potentially confer the benefit of increased aerobic capacity (Mangum 1997; Truchot 1975), high Mg<sup>2+</sup> levels may also have a detrimental effect on muscle function, leading to a decrease in maximal contraction force (Fabiato & Fabiato 1975; Sigel & Sigel 1990). In attackers, this could lead to weaker shell rapping; the power of shell rapping has previously been shown to increase the chance of victory (Briffa & Elwood 2000c). Nevertheless, analyses of the temporal pattern of shell rapping (Briffa & Elwood 2000a; Briffa et al. 1998) suggest that the primary function is as a signal of ‘stamina’ rather than as a demonstration of ‘strength’. Therefore, if there is a potential trade-off between the benefits of increased respiratory capacity against the cost of narcotising effects on muscle tissue, it appears that any loss of muscle power is outweighed by the benefits of enhanced performance capacity. It is clear that this would benefit crabs adopting the attacker role, which perform bouts of vigorous shell rapping. Further, the negative narcotising effects of heightened Mg<sup>2+</sup> presence are likely to be short term (Spicer et al. 1994), effective during the timescale of a contest, whereas the potential positive effects would be medium term, providing benefits during post-contest recovery. Rapid post-fight recovery from oxygen debt would be useful for recommencement of other activities. Further, the avoidance of tissue damage associated with the accumulation of *L*-lactate would be a long term benefit with respect to the cost of cellular regeneration. It may therefore be of benefit to allow activity levels to be somewhat reduced during the course of a contest in order to increase them more quickly afterwards. This trade-off may thus influence strategic decisions to persist in encounters, with the level of mobilised Mg<sup>2+</sup> being based on information gained about the opponent.

In defenders, [Ca<sup>2+</sup>]<sub>HL</sub> was not only highest in winners, but was also shown to correlate with many of the parameters of the pattern of shell rapping (Figures 2.16 - 2.19), and

increased with contest duration, both in terms of time (Figure 2.16) and in terms of the amount of shell rapping inflicted on them by attackers (Figure 2.17). This further supports the possibility that  $[Ca^{2+}]_{HL}$  increases as a consequence of fighting. It is interesting that this increase was only detected in defenders, which appear to perform fewer obviously demanding activities than attackers during shell fights. Furthermore, it is not immediately clear why successful defenders would have elevated levels of circulating metal ions compared to evicted defenders when their main defensive activity is resisting the attacker's attempts at eviction. They remain tightly withdrawn inside the defended shell, and to effectively resist it is presumably necessary to maintain a powerful grip on it using the abdominal musculature. However, aerobic respiration may be restricted within the confines of the shell and the oxygen content of the water available to the defender is likely to fall as the fight progresses. Since  $Mg^{2+}$  and  $Ca^{2+}$  may enhance aerobic capacity, high levels might allow defenders to persist for longer in these encounters. Furthermore,  $Mg^{2+}$  and  $Ca^{2+}$  may also buffer against acidosis incurred due to anaerobic respiration in response to declining oxygen levels inside the shell (DeFur et al. 1980; Henry et al. 1981).

There is also the possibility, for both roles, that the combined beneficial effects of elevated  $Ca^{2+}$  and  $Mg^{2+}$  are greater in magnitude than the detrimental effects of  $Mg^{2+}$  only. Interestingly, defenders were found to have higher  $[Mg^{2+}]_{HL}$  and  $[Ca^{2+}]_{HL}$  than attackers. As the concentration of metal ions was not related to the size of the crabs this indicates that the differing metal ion levels are due to the different roles adopted during the fight. Indeed, in control crabs that did not fight there were no differences in the levels of metal ions between the roles. Previous studies have shown that defenders exhibit elevated rates of glycogen metabolism (Briffa & Elwood 2004), and from the evidence of circulating metal ions it appears that crabs adopting the defender role, as well as those playing attacker, are subject to physiological changes associated with demanding activity.

Circulating levels of metal ions were also found to vary seasonally, although these effects differed between roles (Figures 2.4, 2.7 and 2.10), and outcomes (Figures 2.5, 2.6, 2.11 and 2.13). Like most organisms, hermit crabs are subject to a fluctuating environment, which may induce physiological changes, and therefore alter fighting ability, on a seasonal basis. Seasonal changes in motivation and fighting ability are usually associated with a temporally isolated resource (e.g. food or mates), a primary example being the rut and antler growth in red deer, *Cervus elaphus* (see Clutton-Brock & Albon 1979; Clutton-Brock et al. 1979 and references therein). However, hermit crabs must acquire new gastropod shells as their size increases, and as their growth is not confined to specific seasons they may have to fight at

any time of the year.  $[Mg^{2+}]_{HL}$  showed a strong decrease from spring to summer as the seasons became warmer, although this seasonal change was more marked in defenders than in attackers (Figure 2.5).

Conversely,  $[Ca^{2+}]_{HL}$  increased from spring to summer, and this seasonal difference was more marked in attackers than in defenders (Figure 2.10). It is possible that attackers have a greater need for elevated  $[Ca^{2+}]_{HL}$  as they perform more obviously demanding activities than defenders during shell fights, and a seasonal increase in metabolism mediated by warming would bring about a heightened ability to regulate  $Ca^{2+}$ . Thus, attackers may have a greater need for the tighter regulation of  $Ca^{2+}$ , and are better able to do this in the summer than in the spring. The effect of season on  $[Mg^{2+}]_{HL}$  and  $[Ca^{2+}]_{HL}$  therefore has important implications for each role adopted during the contest.

The beneficial effect of heightened  $[Ca^{2+}]_{HL}$  on the fighting ability of attackers also depends on the season in which the fight takes place (Figure 2.13). It appears that attackers only need to maintain  $[Ca^{2+}]_{HL}$  at the level found in control crabs in order to win contests in the spring, whereas they must elevate  $[Ca^{2+}]_{HL}$  substantially in order to win contests in the summer. As both successful and unsuccessful attackers exhibited elevated  $[Ca^{2+}]_{HL}$  in the summer, but winners having higher  $[Ca^{2+}]_{HL}$  than losers, it appears that they must regulate it at a relatively high level in order to be victorious. This seasonal effect has important implications for the fighting ability of hermit crabs as it appears that poor regulators of  $Ca^{2+}$  are at a greater disadvantage in the spring than in the summer.

The regulation of metal ions is an active and continuous process that is subject to seasonal variation. It also appears that this regulation of metal ions is modified as a consequence of fighting. Moreover, the nature of this modification may be linked to strategic decisions, as winners had higher levels than losers. Magnesium and calcium may thus reduce the costs of being in a fight and alter the threshold associated with the giving up decision. In the context of aggression it now appears that, in addition to their influence on overall activity rates, circulating metal ions may also influence the key strategic decisions that determine contest outcomes. Thus, not only complex compounds such as metabolites and hormones may be of importance during the decision making process, but also very simple substances such as ions.

## **CHAPTER 3**

**The role of haemocyanin concentration on the vigour and outcomes of hermit crab shell fights.**



### ***Abstract***

Strategic decisions in animal contests have been increasingly understood in terms of the physiological consequences of fighting, in particular by investigating energetic costs. In addition to these costs, the scope for vigorous agonistic activity may be modified by the aerobic capacity of engaging individuals, which can be linked to the circulating levels of respiratory pigment. Greater concentrations of respiratory pigment would enhance the aerobic capacity of fighting animals, thus prolonging aerobic respiration, postponing the onset of anaerobia, and reducing the amount of metabolic acidosis incurred. This study examined post-fight levels of the respiratory pigment haemocyanin in the hermit crab *Pagurus bernhardus*. The concentration of haemocyanin was found to be higher in winners than losers, but as the synthesis and catabolism of haemocyanin takes several hours, the concentration of haemocyanin would be expected to change only slightly during the course of a brief contest. Haemocyanin concentration was also found to be positively correlated with the size of the animals; residuals calculated for how much each individual deviated from this relationship revealed that individuals that had more haemocyanin than that predicted for their size (positive residuals) were more likely to win aggressive encounters. Thus, greater levels of respiratory pigment appear to enhance agonistic performance via their influence on endurance and aerobic capacity.

## INTRODUCTION

Aggressive encounters between animals often include the use of signals that are assumed to advertise the fighting ability (RHP) of the sender (Payne & Pagel 1996; Payne & Pagel 1997). Such signals can either be 'conventional' or 'costly'. Although they will incur long-term costs in terms of growth and maintenance, conventional signals are both cheap to perform and receive in the time frame of the interaction (Dawkins & Guilford 1991). They usually involve assessment of cues that reflect opponent quality such as body size (e.g. Caldwell & Dingle 1979; Riechert 1978), weapon size (e.g. Barki et al. 1997; Sneddon et al. 1997) or ornamentation (e.g. Lailvaux et al. 2005; Vanhooydonck et al. 2005). However, these visual assessment criteria may not accurately reflect the current quality of the sender, which may be in poor condition due factors such as recent injury or exhaustion. Thus conventional signals are open to 'cheating' (Dawkins & Guilford 1991) whereby an animal may signal that it is of better quality than it actually is. However, signalling systems that employ costly signals are not generally vulnerable to invasion by cheats (Grafen 1990; Zahavi 1977). Here, an intrinsic cost associated with the production of the signal constrains the level of its performance and thus precludes an animal from signalling at a level beyond its capability (Hurd 1997). Many studies have analysed fights with respect to the proximate physiological consequences of aggressive behaviour in order to measure the costs of agonistic behaviour including signalling. These costs have been demonstrated in terms of endocrine changes (Briffa & Elwood 2007; Sneddon et al. 2000b) and energetic costs (Briffa & Elwood 2001; Briffa & Elwood 2002; Briffa & Elwood 2004; Neat et al. 1998; Schuett & Grober 2000; Sneddon et al. 1999; Thorpe et al. 1995) across a range of taxa. Furthermore, costly signalling often involves the repeated performance of a potentially exhausting activity, and thus reveals the quality of the signaller once it reaches its maximum signalling capacity (Grafen 1990). Studies of repeated signals in a wide range of taxa, including hermit crabs (Briffa et al. 1998), toads (Sullivan & Walsberg 1985), birds (Horn et al. 1995; Weary et al. 1991) and red deer (Clutton-Brock et al. 1979) have indicated a link between the pattern of performance and agonistic success.

The physiological costs associated with such signals are thus expected to accrue as a result of sustaining demanding activity over extended periods. Therefore the ability to avoid these costs for longer would confer an advantage to a displaying individual by prolonging the time that it is able to signal over or increasing the maximum signal intensity that it is able to perform. This physical endurance capacity or stamina thus dictates the duration of demanding

activity an individual can perform before exhaustion or ‘maximum exertion’ (Lailvaux et al. 2005). By advertising its stamina, an individual can demonstrate its ability to meet the physiological demands of engaging in aggressive encounters, and thus its resource holding potential (RHP) (Briffa & Elwood 2001; Clutton-Brock & Albon 1979).

In contrast to such ‘mutual assessment’, models such as the cumulative assessment model (CAM: Payne 1998) and the energetic war of attrition model (E-WOA: Payne & Pagel 1996; Payne & Pagel 1997) assume ‘self-assessment’, where the costs associated with producing a ‘signal’ dictate when the sender should withdraw. There is difficulty in terming such behaviours ‘signals’ when the models assume no mutual assessment. However, individuals presumably still perceive these behaviours and are aware that their opponent is still in competition. The CAM predicts that production costs, together with costs inflicted by the opponent, influence the threshold dictating withdrawal from the fight (Payne 1998). The E-WOA, however, predicts that an opponent should withdraw once the costs associated with producing a signal cross its own physiological threshold. Thus, both models assume that individuals undergo self-assessment, and base their decisions on individual cost thresholds that are based on stamina. However, in the E-WOA model the decision to withdraw is based on the accumulation of costs due solely to the competitor’s own actions only, and the contest ends when the losing contestant reaches its own threshold level of energetic costs (Smallegange et al. 2007; Taylor & Elwood 2003).

Stamina can be more formally described as ‘the capacity to perform a behaviour at a specific rate over a prolonged period’ or ‘resistance to the accumulation of energetic costs’ and repeated signals may involve demanding activity in order to incur these costs and thereby demonstrate stamina (Payne & Pagel 1996; Payne & Pagel 1997). The two key physiological mechanisms contributing to stamina are the energy reserves and aerobic capacity available to an individual. Energetic expenditure causes energy reserves to be mobilised and decline as they are used. Furthermore, challenging and exceeding aerobic capacity results in anaerobic respiration. This is less efficient than aerobic respiration, producing less ATP per molecule of glucose (Sadava et al. 2008) and results in the accumulation of *L*-lactate (for reviews see Bridges 2001; Briffa & Sneddon 2007).

An individual’s ability to withstand, and avoid, the consequences of anaerobic respiration may therefore become an important factor contributing to stamina, and postponing the onset of anaerobia (e.g. by increasing aerobic capacity) may enhance endurance. Thus, the aerobic capacity of individuals may be important in determining the outcome of fights, and may be demonstrated by agonistic signals that directly constrain respiration, e.g. lateral displays in

male side-blotched lizards *Uta stansburiana* (Brandt 2003), and opercular flares in Siamese fighting fish *Betta splendens* (Abrahams et al. 2005) as well as by displays of endurance.

The aerobic capacity and thereby stamina of an animal will be affected by the circulating concentration of oxygen transporting respiratory proteins in the blood. For example, Kobayashi and Gonoï (1985) showed that the swimming ability of *Daphnia magna* held in hypoxic water was directly proportional to their haemoglobin concentration. Thus, greater concentrations of respiratory pigment could enhance the aerobic capacity of fighting animals, prolonging aerobic respiration, postponing the onset of anaerobia, and reducing the metabolic costs incurred during encounters.

Experimental manipulation of the oxygen concentration available to fighting animals has been shown to affect the build up of *L*-lactate and the outcome of aggressive encounters in shore crabs *Carcinus maenas* (Sneddon et al. 1998) and hermit crabs *Pagurus bernhardus* (Briffa & Elwood 2000b). Indeed, crustaceans in particular seem unable to sustain aerobic respiration for long periods of exercise, possibly due to diffusion limitations associated with calcified gill structures and extracting oxygen from an aquatic environment (Booth et al. 1982). Furthermore, *L*-lactate levels in crustaceans remain elevated for up to 24 h after periods of exercise (Booth et al. 1982), suggesting that crustaceans are unable to metabolise it quickly. Thus, avoiding anaerobic respiration is not just important within the timescale of a contest, but potentially for up to a day afterwards as well. The ongoing effects of *L*-lactate-mediated fatigue could thereby continue to constrain the ability to perform other activities (e.g. foraging) and it would therefore be of benefit to avoid or postpone anaerobia as much as possible. This is likely to be determined by aerobic capacity, which should vary with oxygen transport capacities. In vertebrates, differences in oxygen transport capacities are most likely to be governed by differences in the condition of their closed circulatory systems, whereas in invertebrates, variation in the concentration of circulating respiratory pigment would be more important in contributing to variation in oxygen transport (see Sadava et al. 2008).

The major respiratory pigment of most crustaceans is haemocyanin (Hc, Truchot & Lallier 1992), which is a freely circulating copper-based respiratory protein (Redfield 1934; Redmond 1955; Terwilliger 1998), accounting for up to 97% of oxygen exchange in crustaceans (Redmond 1955). The ability of Hc to transport oxygen may be modulated by several allosteric factors present in the haemolymph (for reviews see Bridges 2001; Mangum 1990), some of which may affect fighting ability, e.g. monoamines (Briffa & Elwood 2007; Sneddon et al. 2000b) and the metal ions  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Mowles et al. 2008, Chapter 2). However, a more direct process by which to increase aerobic capacity would be the synthesis

of greater quantities of Hc (Terwilliger 1998). For example, exposure to sublethal environmental hypoxia induces the synthesis of additional Hc (DeFur et al. 1990; Hagerman 1986; Spicer & Baden 2001), increasing the oxygen carrying capacity of the blood to an appropriate value over approximately 24 hours (Spicer & Baden 2001). Conversely, starvation results in a decrease in blood Hc concentrations ( $[Hc]_{HL}$ ), indicating that Hc may serve the dual function of respiratory pigment and storage protein, being catabolised to release energy in times of need (Baden et al. 1994; Hagerman 1986; Redmond 1955).

Due to the temporal plasticity of  $[Hc]_{HL}$ , as well as the differing intrinsic capacities of individuals to synthesise it, there is a high degree of intra-specific variability in its concentration (Redmond 1955; Spicer & Baden 2000; Spicer & Baden 2001), which is likely to influence fighting ability. Furthermore, because the synthesis and catabolism of Hc, is not immediate, its concentration should remain relatively stable over the duration of a contest. Hc thus represents a physiological variable that may ultimately predispose individuals to perform at a particular level during aggressive encounters, through its affect on 'stamina'.

Fights between hermit crabs commonly occur over the ownership of the gastropod shells that they occupy. During these shell fights, the opponents adopt two very different roles. The initiating crab or 'attacker' attempts to take the shell of the non-initiator or 'defender' following an assessment period comprising bouts of 'shell-rapping', during which the defender remains tightly withdrawn inside its shell. The attacker grasps the defender, gripping the contested shell with its walking legs, and proceeds to bring its own shell repeatedly into contact with that of the defender in quick succession as a bout of raps. The shell fight consists of a series of such bouts, separated by distinct pauses. Shell fights can end in one of two ways: either the defender decides to give up, allowing itself to be evicted by the attacker, which removes the defender through the aperture of the shell; or the attacker may decide to give up first without having evicted the defender.

The vigour of shell-rapping is a key determinant of the outcome of shell fights, and it has been consistently shown that successful attackers perform more raps per bout, leave shorter pauses between bouts of rapping (Briffa et al. 1998), and perform more powerful raps (Briffa & Elwood 2002). This suggests that the pattern of rapping contains information about the fighting ability of the attacker, acting as an honest signal (Briffa 2006), which advertises its stamina (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998). The honesty of the signal is maintained as it is costly to perform, resulting in the accumulation of metabolic by-products (e.g. L-lactate, Briffa & Elwood 2001) and depletion of energy reserves (e.g. glycogen, Briffa & Elwood 2004). Thus, the metabolic consequences of performing

demanding signals may impose physiological limitations to behaviour, thereby precluding an animal from signalling beyond its capacity. Furthermore, the stamina of an animal may be influenced by its aerobic capacity, which would limit the degree of activity possible before anaerobic respiration takes effect. The aim of this study was therefore to examine how circulating concentrations of respiratory pigment influence contest behaviour, particularly in terms of strategic decisions and fight vigour, using hermit crab shell fights and Hc as a model system.

## MATERIALS AND METHODS

### *Study organisms*

Specimens of the common European hermit crab *Pagurus bernhardus* were collected between December 2006 and March 2007 from rock pools at Hannafore Point, UK (50° 20' N, 4° 27' W). The crabs were restricted to a size range sufficient for the extraction of 20 µl haemolymph samples (crab weight = 0.590-1.930 g).

The hermit crabs were kept in groups of 80-150 in 65cm x 50cm x 30cm plastic tanks, filled with aerated seawater to a depth of 15cm (~50 l). These were maintained at 15°C in a temperature controlled laboratory. The crabs were fed on a diet of catfish pellets and were returned to the shore within one month of collection.

### *Staging fights and recording behaviour*

Hermit crabs were removed from their gastropod shells by cracking the shells open in a bench vice. They were then sexed and only male crabs were used in order to avoid sex-related behavioural differences as found in previous studies (Neil & Elwood 1985). Study animals were further discriminated on the basis of physical damage, and only crabs which were (i) free from obvious parasites, (ii) undamaged and (iii) intermoult were used in the experimental trials. Unused crabs were provided with new shells and were returned to the site from which they were originally collected.

The study crabs were assigned to pairs consisting of a small crab and a large crab (mean weights  $\pm$  s.e.; small,  $0.797 \pm 0.013$  g; large,  $1.064 \pm 0.021$  g). The larger crab of the pair (potential attacker) was provided with a *Littorina littorea* shell that was only 50% of its preferred shell weight, while the smaller crab (potential defender) was provided with a *L. littorea* shell which would be 100% adequate for the larger crab of the pair. The preferred shell size was determined using a regression equation that related crab weight to preferred shell weight (Briffa & Elwood 2007).

After shell allocation, crabs were isolated in 95 mm diameter crystallizing dishes filled with aerated seawater, and allowed to acclimate for 16 hours prior to being used in fights. Encounters were staged in two groups to which the pairs of crabs were randomly allocated. In the control group ( $N = 21$  pairs), haemolymph samples were taken from crabs where the

fight was terminated as the attacker made initial contact with the shell of the defender. This process ensured that control samples were being taken from healthy crabs that were prepared to fight. The second group ( $N = 95$  pairs) comprised fights that were allowed to proceed until they concluded, where either the defender allowed itself to be evicted from its shell ( $N = 60$  pairs); or the attacker terminated the fight without first having evicted the defender ( $N = 35$  pairs).

Following Briffa and Elwood (2000a), five fights were staged each day between 0830 and 1330 hours. A large crab was placed in an arena consisting of a 95 mm diameter crystallizing dish containing a 1 cm thick sandstone tile (to provide grip for the pereopods) as a base, and filled to a depth of 4 cm with aerated seawater. The dish was then placed in an observational chamber, behind a two-way mirror, so that the crabs could not see the observer. The small crab was then introduced into the arena containing the large crab and their ensuing behaviours recorded (see ethogram in Chapter 2 Materials and Methods) using a Psion Workabout MX (Psion Teklogix Inc., Mississauga, ON, Canada) configured as a time-event recorder using The Observer 5.0 (Noldus IT, Wageningen, The Netherlands) event-recording software. The parameters of the pattern of shell-rapping (contest duration in seconds, total number of raps, total number of bouts of rapping, mean number of raps/bout and mean pause duration in seconds) were then analysed in order to calculate the vigour of the pattern of shell rapping performed by attackers for each fight (see ethogram in Chapter 2 Materials and Methods).

### *Analysis of $[Hc]_{HL}$*

Immediately after the fight, the crabs were removed from their shells, and haemolymph samples (approx. 20  $\mu$ l) were removed from each crab using a syringe (BD Micro-Fine™ 1mL, Becton, Dickinson and Company, NJ, USA), the needle of which (29 G) was inserted into the infrabranchial sinus, via the arthrodial membrane at the base of the third pereopod. The  $[Hc]_{HL}$  concentration was estimated using a well-established spectrophotometric method (Nickerson & Van Holde 1971; Spicer & Baden 2000). Each sample was aerated by running it through a Microliter syringe™ (Hamilton, Bonaduz, Switzerland) twice in order to ensure that the Hc was fully oxygenated. The samples were then diluted by a factor of 20 by adding 380  $\mu$ l ultra pure water (Elga Maxima, High Wycombe, UK) to each one.



The absorbance of the resultant mixture was measured at 337.0 nm using a spectrophotometer (Unicam Heλios β, Cambridge, UK) with a 2 nm bandwidth. The [Hc]<sub>HL</sub> was then determined using the extinction coefficient (E) calculated by Nickerson and Van Holde (1971):

$$E_{1\text{cm}}^{\text{mMol.l}^{-1}} = 17.26$$

Following this formula, the total absorbance of the solution divided by 17.26 gives the concentration of haemocyanin in mMol/L. This extinction coefficient allows the measurement of a sample against a blank, rather than against a calibration curve. As calibration with copper would result in the unwanted inclusion of free copper, the extinction coefficient facilitates the accurate estimation of [Hc]<sub>HL</sub> alone.

RESULTS

EFFECT OF FIGHTING

A repeated measures ANOVA was performed in order to test whether the [Hc]<sub>HL</sub> was affected by fighting. The between subject factor in the model was fight status (fought or the unfought control group), while the repeated measure was the [Hc]<sub>HL</sub> in each role (attacker or defender).

There was no significant difference in [Hc]<sub>HL</sub> between the unfought control group and crabs that fought ( $F_{1,113} = 2.136, P = 0.1466$ ), and no significant interaction between the two roles and whether they fought ( $F_{1,113} = 0.670, P = 0.4146$ ; Figure 3.1). Attackers, however, had higher [Hc]<sub>HL</sub> than defenders ( $F_{1,113} = 4.540, P = 0.0353$ ; Figure 3.1).

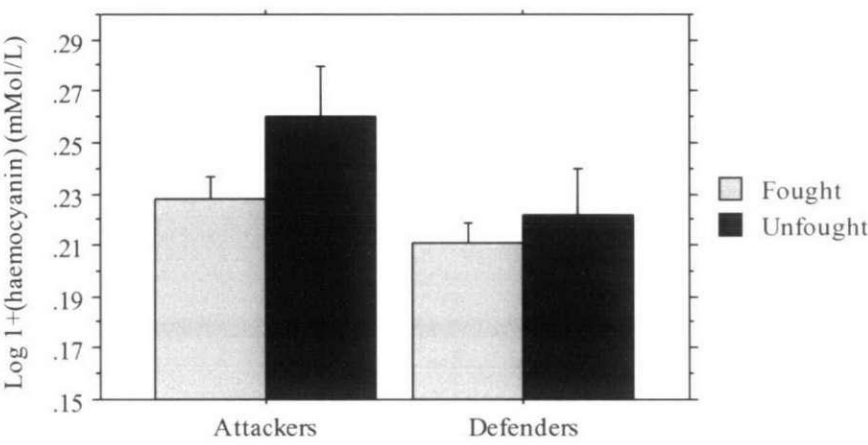
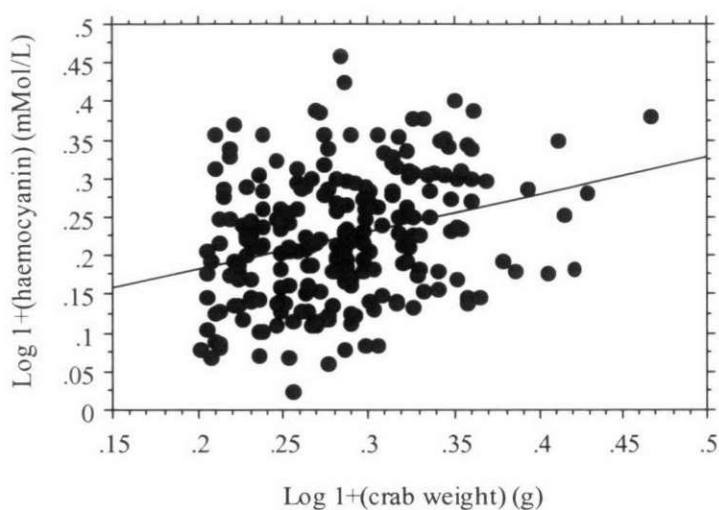


Figure 3.1. [Hc]<sub>HL</sub> in each role in relation to whether the crabs fought.

As attackers are larger than defenders, this difference could have been driven by the size of the crabs. In order to test whether this was the case, an ANCOVA was performed using the data for all of the crabs, which included the [Hc]<sub>HL</sub> as the dependent variable, and the weight of the crabs as a covariate. The fight status of the crabs (fought or the unfought control group) was included as a factor in the model. No significant interaction effect was detected, and this was deleted from the model. There was no difference between the [Hc]<sub>HL</sub> of the fought crabs and the unfought control group ( $F_{1,228} = 0.870, P = 0.3520$ ). However, a significant positive relationship was detected between the weight of the crab and the [Hc]<sub>HL</sub> ( $F_{1,228} = 19.753, P < 0.0001$ ; Figure 3.2).



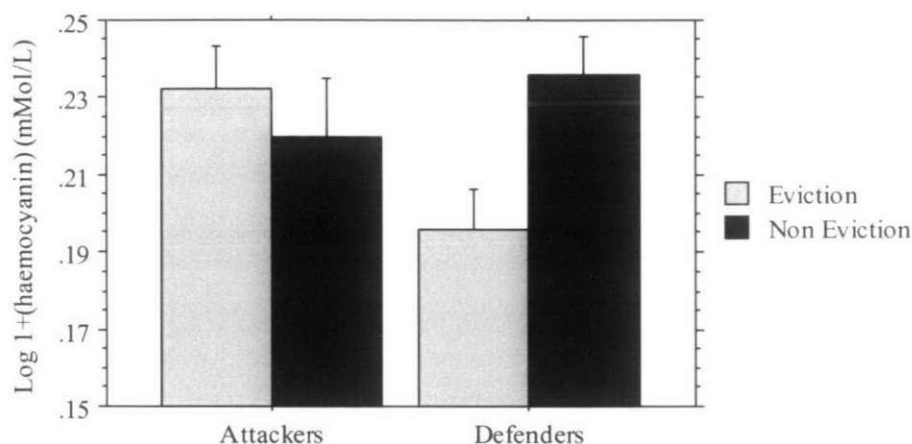
**Figure 3.2.**  $[Hc]_{HL}$  in relation to crab weight.

### EFFECT OF FIGHT OUTCOMES

#### *Repeated Measures ANOVA – Differences in $[Hc]_{HL}$ between roles and outcomes*

The relationships between Hc and fight outcome were determined by performing a repeated measures ANOVA. The between subject factor was outcome (eviction or non-eviction), while the repeated measure was the  $[Hc]_{HL}$  in each role (attacker or defender).

There was no significant difference in the  $[Hc]_{HL}$  between the fight outcomes ( $F_{1,92} = 1.117$ ,  $P = 0.2934$ ), or between the roles adopted by the crabs ( $F_{1,92} = 0.906$ ,  $P = 0.3436$ ). However, a significant interaction was found between the role adopted by the crab and the fight outcome ( $F_{1,92} = 5.851$ ,  $P = 0.0175$ ; Figure 3.3). Attackers that effected evictions had higher  $[Hc]_{HL}$  than attackers that did not evict defenders, whereas non-evicted defenders had higher  $[Hc]_{HL}$  than defenders that were evicted. Thus, in both roles,  $[Hc]_{HL}$  was greater in victorious crabs.



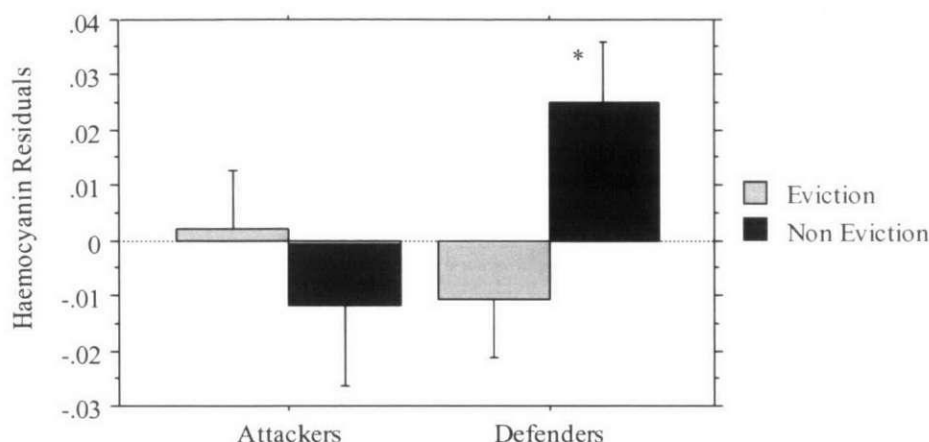
**Figure 3.3.**  $[Hc]_{HL}$  in each role in relation to the outcome of the fight.

### *Repeated Measures ANOVA – Differences in Hc residuals between roles and outcomes*

As  $[Hc]_{HL}$  was found to be positively correlated with the weight of the crabs (Figure 3.2), the residual  $[Hc]_{HL}$  from this relationship were calculated in order to investigate the extent to which  $[Hc]_{HL}$  deviated from this relationship between roles and outcomes. Residuals were calculated from a simple regression with the log of the  $[Hc]_{HL}$  as the dependent variable, and the log of the crab weight as the independent variable. Individuals with positive residuals had a greater  $[Hc]_{HL}$  than the level predicted for their size, while individuals with negative residuals had a lower  $[Hc]_{HL}$  than that predicted for their size.

The effects of Hc residuals on fight outcome were determined by performing a repeated measures ANOVA. The between subject factor in the model was outcome (eviction or non-eviction), while the repeated measure was the Hc residual for each role (attacker or defender).

There was no difference in the Hc residuals between the fight outcomes ( $F_{1,92} = 0.728$ ,  $P = 0.3958$ ), or between the roles adopted by the crabs ( $F_{1,92} = 1.211$ ,  $P = 0.2740$ ). However, a significant interaction was found between the Hc residuals in each role in relation to fight outcome ( $F_{1,92} = 5.219$ ,  $P = 0.0246$ ; Figure 3.4). Attackers that effected evictions had positive Hc residuals whereas attackers that did not evict defenders had negative Hc residuals. Similarly, non-evicted defenders had positive Hc residuals, whereas defenders that were evicted had negative Hc residuals. Thus, as in the case of raw  $[Hc]_{HL}$ , weight-corrected  $[Hc]_{HL}$  was also greater in victorious crabs for both roles.



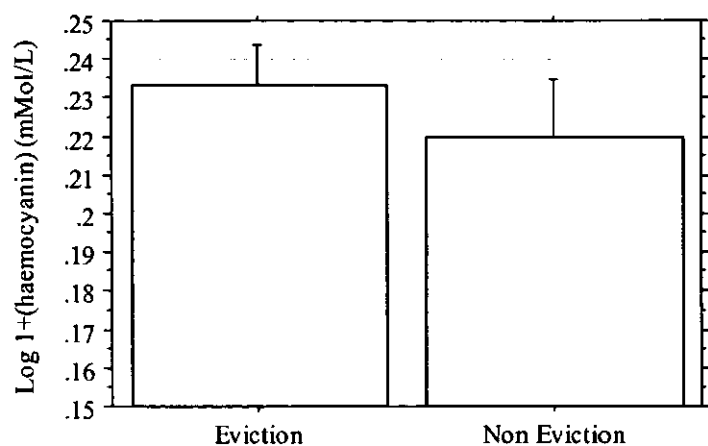
**Figure 3.4.** Hc residuals in each role in relation to the outcome of the fight. \* = Significantly different from zero.

One-sample t-tests were performed for each outcome in each role in order to determine whether the residuals were significantly different from zero. Attackers that effected evictions ( $t = 0.281$ ,  $P = 0.7795$ ,  $df = 59$ ), attackers that did not effect evictions ( $t = -0.813$ ,  $P = 0.4217$ ,  $df = 34$ ) and evicted defenders ( $t = -1.023$ ,  $P = 0.3107$ ,  $df = 58$ ) did not have  $[Hc]_{HL}$  that significantly differed from those predicted for their size. The residuals for non-evicted defenders however, were significantly greater than zero ( $t = 2.312$ ,  $P = 0.0270$ ,  $df = 34$ ; Figure 3.4). Thus, successful defenders that resisted eviction had a higher  $[Hc]_{HL}$  than predicted for their size.

#### ***ANCOVA – Hc in Attackers only***

Interaction effects may mask differences between outcomes within each role. As repeated measures ANOVAs do not allow post-hoc comparisons between specific groups, one-factor ANCOVAs were used to examine each role independently. The  $[Hc]_{HL}$  was the dependent variable in these models, while the weight of the crabs was the covariate. The factor included in the model was fight outcome (eviction or non-eviction).

When attackers were analyzed on their own, no significant interaction effects were detected, and were thus deleted from the model. There was no significant difference in the  $[Hc]_{HL}$  between outcomes ( $F_{1,92} = 0.785$ ,  $P = 0.3780$ ; Figure 3.5).



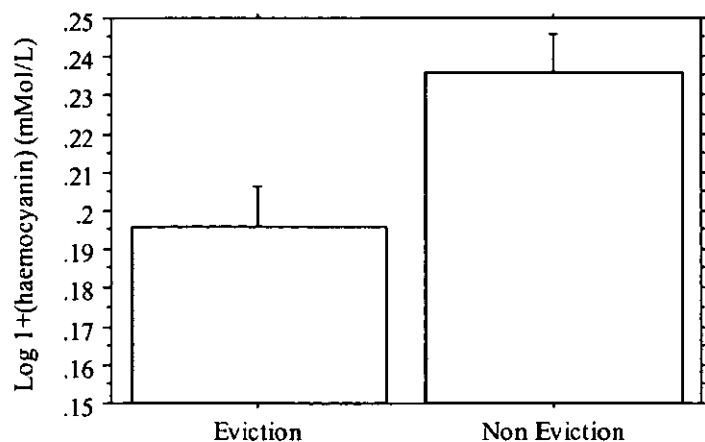
**Figure 3.5.**  $[Hc]_{HL}$  in attackers in relation to fight outcome.

However, a significant relationship was detected between the  $[Hc]_{HL}$  and the weight of the attackers, with  $[Hc]_{HL}$  increasing with the weight of the crabs ( $F_{1,92} = 8.607$ ,  $P = 0.0042$ ).

#### ***ANCOVA – Hc in Defenders only***

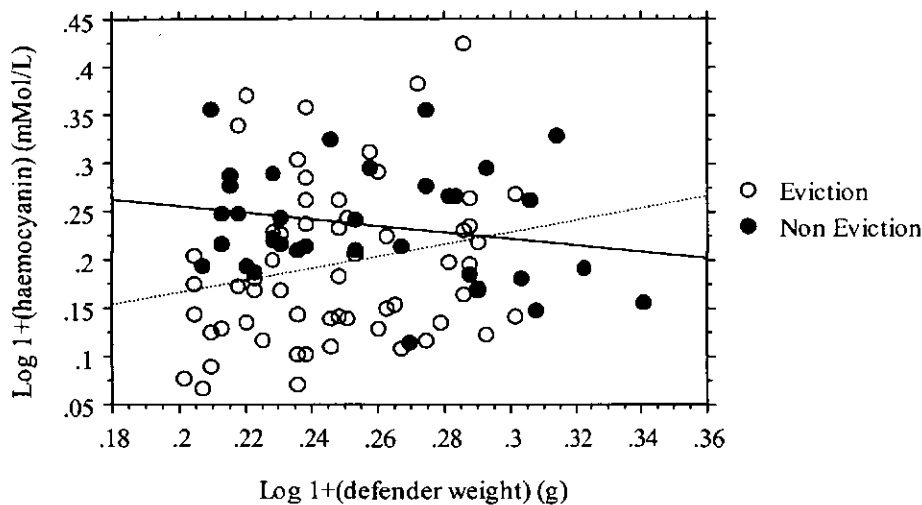
Again, in order to investigate possible interaction effects, defenders were analysed independently of attackers using a one-factor ANCOVA. The  $[Hc]_{HL}$  was the dependent variable, while the weight of the crabs was the covariate. The factor included in the model was fight outcome (eviction or non-eviction).

When defenders were analysed on their own, a significant difference was detected in the  $[Hc]_{HL}$  between the fight outcomes, with non-evicted defenders having greater levels than evicted defenders ( $F_{1,90} = 5.338$ ,  $P = 0.0232$ ; Figure 3.6).



**Figure 3.6.**  $[Hc]_{HL}$  in defenders in relation to fight outcome.

Unlike attackers, there was no significant relationship between the  $[Hc]_{HL}$  and the weight of defenders ( $F_{1,90} = 0.386$ ,  $P = 0.5360$ ). However, there was a significant interaction effect between the outcome of the fight and the weight of the defender ( $F_{1,90} = 4.046$ ,  $P = 0.0473$ ; Figure 3.7).



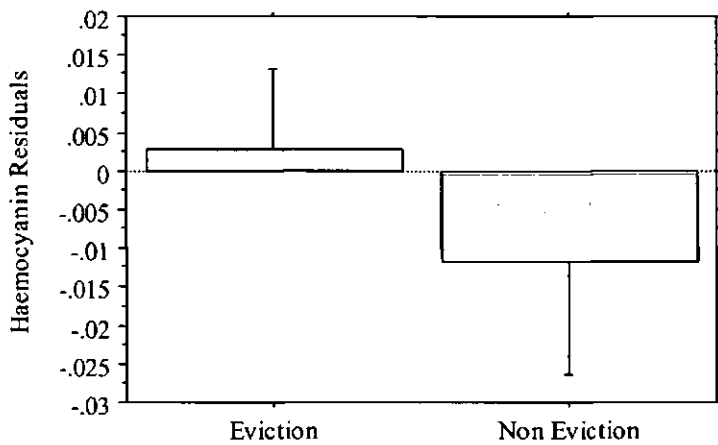
**Figure 3.7.**  $[Hc]_{HL}$  in relation to defender weight.

In evicted defenders, there was a non-significant positive relationship between  $[Hc]_{HL}$  and defender weight ( $R^2 = 0.046$ ,  $F_{1,57} = 2.758$ ,  $P = 0.1022$ ), whereas in non-evicted defenders, there was a non-significant negative relationship between  $[Hc]_{HL}$  and defender weight ( $R^2 = 0.046$ ,  $F_{1,33} = 1.584$ ,  $P = 0.2171$ ).

### ***ANOVA – Hc residuals in Attackers only***

Similar to the raw Hc data, interaction effects may be masking differences within each role in terms of the Hc residuals. In order to investigate this possibility, one-factor ANOVAs were again used to examine each role independently. The Hc residual was the dependent variable in these models and the factor included in the model was fight outcome (eviction or non-eviction). Crab weight was not used as a covariate in this model as it is already included in the calculation of the Hc residuals.

When attackers were analysed on their own, there was no significant difference in the Hc residuals between fight outcomes ( $F_{1,93} = 0.715$ ,  $P = 0.3999$ ; Figure 3.8).

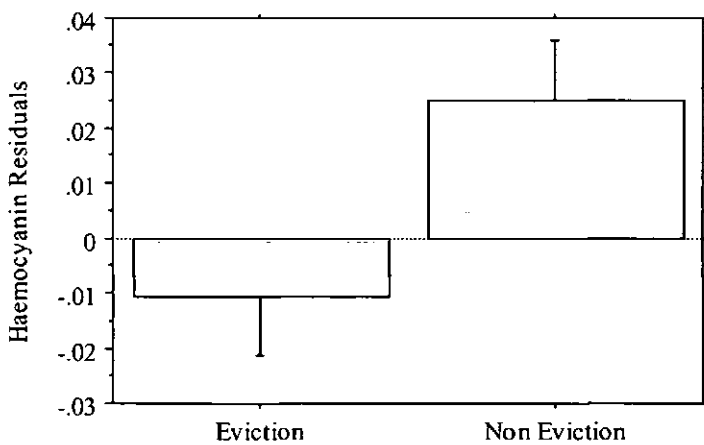


**Figure 3.8.** Hc residuals in attackers in relation to fight outcome.

### ***ANOVA – Hc residuals in Defenders only***

Again, in order to investigate possible interaction effects, defenders were analysed independently from attackers using a one-factor ANOVA. The Hc residual was the dependent variable, while the factor included in the model was fight outcome (eviction or non-eviction).

When defenders were analysed independently from attackers, a significant difference was detected between the fight outcomes, with non-evicted defenders having positive residuals, whereas evicted defenders had negative residuals ( $F_{1,92} = 4.999$ ,  $P = 0.0278$ ; Figure 3.9).



**Figure 3.9.** Hc residuals in defenders in relation to fight outcome.



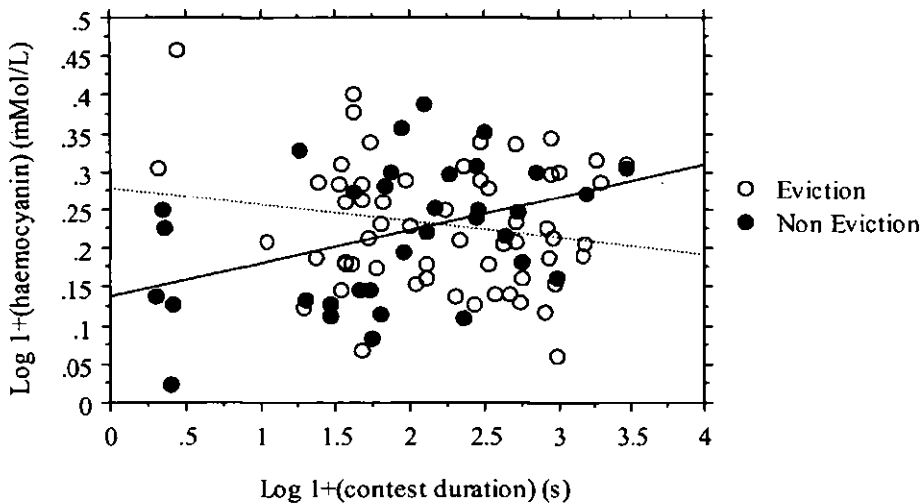
## CORRELATIONS BETWEEN FIGHT VIGOUR AND $[Hc]_{HL}$

### *$[Hc]_{HL}$ concentration and fight vigour*

As  $[Hc]_{HL}$  was unlikely to change during the duration of a fight, and was not demonstrated to be affected by fighting ( $F_{1,113} = 2.136$ ,  $P = 0.1466$ ; Figure 3.1), I assumed that  $[Hc]_{HL}$  may influence fight vigour.

In order to determine the effects of  $[Hc]_{HL}$  on fight vigour, a series of one-factor ANCOVAs were performed which included the  $[Hc]_{HL}$  as the dependent variable, and fight variables associated with vigour as covariates (see ethogram in Chapter 2 Materials and Methods). Fight outcome was included as a factor in the model as it has been shown in the previous section to be influenced by the  $[Hc]_{HL}$  present. Bivariate scattergrams with regression lines have been presented in order to illustrate the direction of the effects.

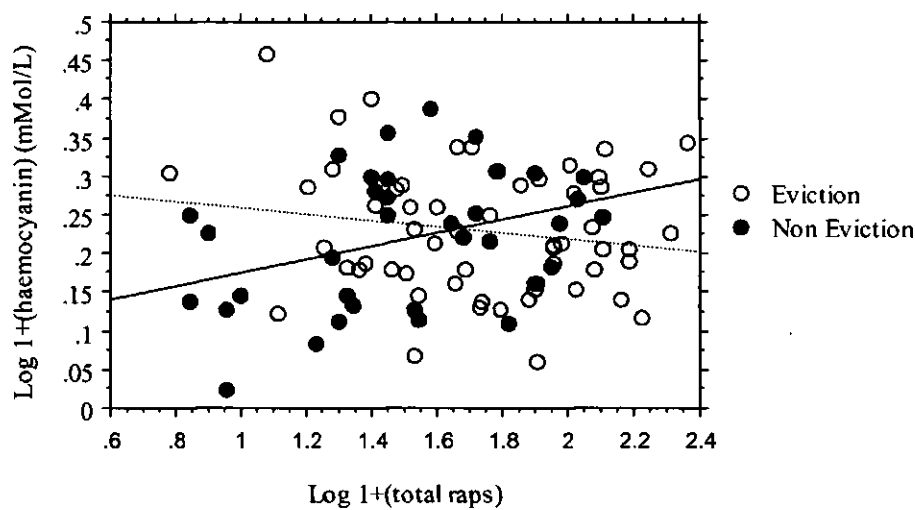
In attackers, there was no significant relationship between  $[Hc]_{HL}$  and contest duration ( $F_{1,90} = 0.901$ ,  $P = 0.3451$ ), but a significant interaction was detected between the outcome of the contest and the contest duration ( $F_{1,90} = 7.981$ ,  $P = 0.0058$ ; Figure 3.10). There was a non-significant trend for contest duration to decrease with  $[Hc]_{HL}$  in attackers that effected evictions ( $R^2 = 0.033$ ,  $F_{1,57} = 1.965$ ,  $P = 0.1664$ ), whereas contest duration increased with  $[Hc]_{HL}$  in those attackers that did not effect evictions ( $R^2 = 0.164$ ,  $F_{1,33} = 6.451$ ,  $P = 0.0160$ ).



**Figure 3.10.**  $[Hc]_{HL}$  in attackers in relation to contest duration and the outcome of the contest.

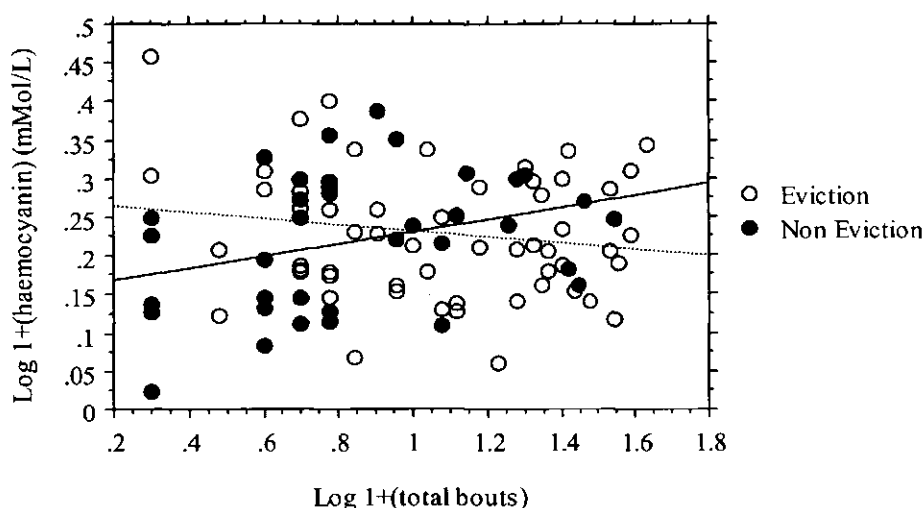
There was also no significant relationship between  $[Hc]_{HL}$  and the total number of raps in the contest ( $F_{1,90} = 0.885$ ,  $P = 0.3494$ ). However, there was a significant interaction between the

outcome of the contest and the total number of raps performed by attackers ( $F_{1,90} = 6.476$ ,  $P = 0.0126$ ; Figure 3.11). In attackers that effected evictions, there was a non-significant trend for  $[Hc]_{HL}$  to decrease with the number of raps performed ( $R^2 = 0.029$ ,  $F_{1,57} = 1.678$ ,  $P = 0.2004$ ), but in attackers that did not effect evictions, the  $[Hc]_{HL}$  increased with the total number of raps ( $R^2 = 0.128$ ,  $F_{1,33} = 4.858$ ,  $P = 0.0346$ ).



**Figure 3.11.**  $[Hc]_{HL}$  in attackers in relation to the total number of raps and the outcome of the contest.

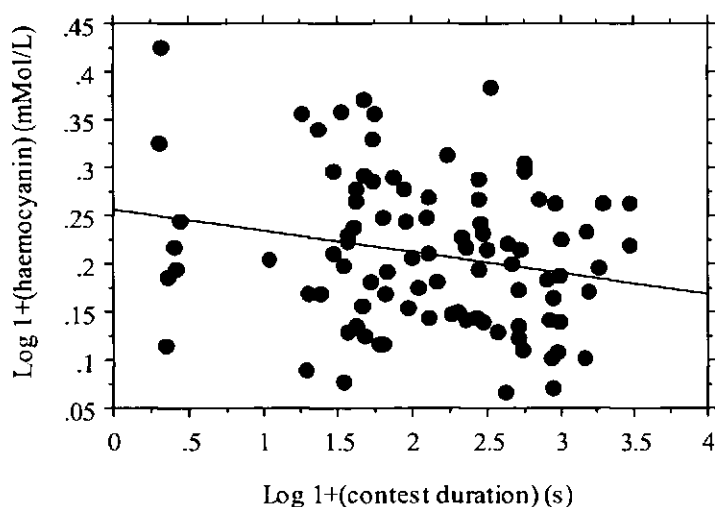
Similarly, there was no significant relationship between  $[Hc]_{HL}$  and the total number of bouts in the contest ( $F_{1,90} = 0.568$ ,  $P = 0.4528$ ). However, there was a significant interaction between the outcome of the contest and the total number of bouts of rapping performed by attackers ( $F_{1,90} = 5.662$ ,  $P = 0.0194$ ; Figure 3.12). There was a non-significant trend for  $[Hc]_{HL}$  to decrease with the number of bouts performed by attackers that effected evictions ( $R^2 = 0.030$ ,  $F_{1,57} = 1.791$ ,  $P = 0.1861$ ), while there was a non-significant trend for it to increase with the number of bouts performed by attackers that did not effect evictions ( $R^2 = 0.103$ ,  $F_{1,33} = 3.788$ ,  $P = 0.0602$ ).



**Figure 3.12.**  $[Hc]_{HL}$  in attackers in relation to the total number of bouts and the outcome of the contest.

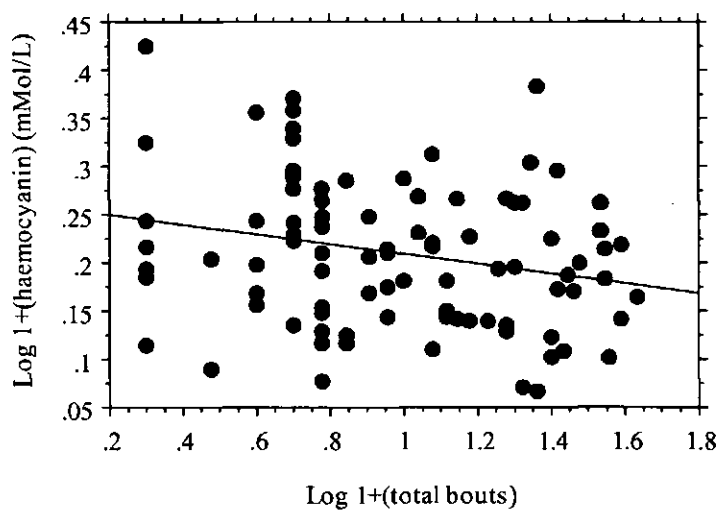
When analysing relationships between the  $[Hc]_{HL}$  and the number of raps/bout and the mean pause duration, there were no interactions involving the covariate, and these were thus deleted from the models, which were recalculated. There was no relationship between the  $[Hc]_{HL}$  and the number of raps/bout ( $F_{1,91} = 0.306$ ,  $P = 0.5816$ ), or between  $[Hc]_{HL}$  and the mean pause duration ( $F_{1,85} = 0.031$ ,  $P = 0.8610$ ).

In defenders, there were no interactions involving the covariate. These were thus deleted and the models recalculated. There was a non-significant trend for  $[Hc]_{HL}$  to decrease with contest duration ( $F_{1,90} = 2.940$ ,  $P = 0.0899$ ; Figure 3.13).



**Figure 3.13.**  $[Hc]_{HL}$  in defenders in relation to contest duration.

No significant relationship was found between the  $[Hc]_{HL}$  in defenders and the total number of raps in the contest ( $F_{1,90}=2.608$ ,  $P=0.1098$ ), but there was a non-significant trend for  $[Hc]_{HL}$  to decrease with the total number of bouts in the contest ( $F_{1,90}=3.107$ ,  $P=0.0814$ ; Figure 3.14).



**Figure 3.14.**  $[Hc]_{HL}$  in defenders in relation to the total number of bouts.

Similarly to attackers, no relationship was detected between the  $[Hc]_{HL}$  and the number of raps/bout ( $F_{1,90}=1.730$ ,  $P=0.1918$ ), or between  $[Hc]_{HL}$  and the mean pause duration ( $F_{1,84}=0.2491$ ,  $P=0.1183$ ).

## DISCUSSION

Circulating levels of Hc did not change during the course of shell fights (Figure 3.1). They were, however, found to be higher in winners than in losers (Figure 3.3). The circulating  $[Hc]_{HL}$  was also found to vary with the role adopted by the two crabs, being higher in attackers than in defenders. This may be a function of size as attackers were always larger than defenders, and  $[Hc]_{HL}$  was also found to be positively correlated with the size of the crab (Figure 3.2). Residuals calculated from this relationship revealed that successful individuals have greater concentrations of Hc than those predicted for their size (Figure 3.4). High  $[Hc]_{HL}$  appears to be of particular importance in the case of defenders as defenders with positive residuals were most likely to retain their shells. Hc concentration was also found to be positively correlated with the vigour of attackers that gave up (Figures 3.10, 3.11 and 3.12), demonstrating that greater concentrations and larger residuals allow enhanced persistence before the giving up decision is triggered. Conversely, non-significant negative trends were found between the  $[Hc]_{HL}$  of defenders, and the vigour of the fight (Figures 3.13 and 3.14). A possible explanation for this is that the catabolism of Hc as an emergency energy reserve may have already begun. The results thus demonstrate that greater concentrations of Hc are beneficial for fighting hermit crabs, and it is most likely that this is because they serve to enhance aerobic capacity.

Shell fighting involves two distinct roles: attacker and defender, and it is the attacking crab that performs the most obviously demanding behaviour by engaging in shell-rapping. Indeed, failure to effect evictions is associated with elevated *L*-lactate in attackers, whereas giving up in defenders appears to be associated with failure to mobilise glycogen reserves if they perceive a strong attacker (although *L*-lactate does increase in defenders as well) (Briffa & Elwood 2001; Briffa & Elwood 2004). Thus, attackers undergoing more anaerobic respiration generate more *L*-lactate, and are more likely to give up than attackers that can sustain aerobic respiration for longer. This is supported by the interactions illustrated in Figures 3.3 and 3.4, where winners have greater  $[Hc]_{HL}$  than losers. However, no difference was detected between successful and unsuccessful attackers when they were analysed separately from defenders (Figures 3.5 and 3.8). Successful defenders on the other hand had higher  $[Hc]_{HL}$  than defenders that were evicted (Figure 3.4 legend and Figures 3.6 and 3.9). It thus appears that  $[Hc]_{HL}$  is more important to defenders than attackers, despite defenders performing the less obviously demanding of the two roles during shell fights. Once attacked, the defender remains tightly withdrawn inside its shell for the duration of the encounter.

Despite appearing to be relatively inactive, this behaviour is likely to constrain ventilation, and presumably the oxygen content of the water within the shell will fall. Having greater quantities of respiratory pigment, which would enhance aerobic potential, would thus potentially benefit the defenders to a greater extent than attacking crabs. This is in contrast to the results of a previous study where hermit crabs were held in low oxygen water prior to being used in staged fights. Both the vigour of rapping performed by attackers and the likelihood of eviction were reduced after the attacker was held in low oxygen water whereas the low oxygen treatment did not influence the defenders' chances of winning (Briffa & Elwood 2000b).

The concentration of Hc was also found to be positively related to the size of the crabs (Figure 3.2). Although there is no known correlation between size and  $[Hc]_{HL}$  for many crustaceans, in some species they do appear to be related. For example, Abdennour (1997) found positive relationships between  $[Hc]_{HL}$  and body size in the prawns *Palaemon elegans* and *P. longirostris*, but not in *Palaemonetes varians* or *Crangon crangon*. Similarly, Spicer & Baden (2000) found no relationship between size and  $[Hc]_{HL}$  for the Norway lobster *Nephrops norvegicus* or the swimming crab *Liocarcinus depurator*, whilst the size and  $[Hc]_{HL}$  of the spider crab *Hyas araneus* were found to be negatively related. It is not known why such trends exist, but it is likely that larger individuals would have the competitive advantage in acquiring the nutritional resources necessary to synthesise additional Hc, leading to positive relationships. Furthermore, higher concentrations of Hc may be of more benefit to larger individuals due to the diffusion constraints associated with transporting oxygen in a larger body, thus requiring greater concentrations of respiratory pigment for oxygen transport.

Haemocyanin concentration is highly variable between individuals, and this is likely to reflect their recent respiratory and nutritional history, as well as their intrinsic capacity to synthesise Hc (Redmond 1955; Spicer & Baden 2000; Spicer & Baden 2001). Thus, in addition to responses to environmental variables, individuals with greater  $[Hc]_{HL}$  and positive residuals are likely to be better quality individuals, in better condition, and with the anabolic capacity to synthesise this protein. Haemocyanin is a complex protein that requires time and energy to synthesise, and energy is released upon its breakdown. As the catabolism of Hc is also a slow process, the  $[Hc]_{HL}$  was not expected to change during the course of shell fights. However, non-significant negative trends were found between the  $[Hc]_{HL}$  of defenders, and the vigour of shell fights. As defenders have been shown to mobilise energy reserves following a decision to resist attackers (Briffa & Elwood 2001; Briffa & Elwood 2004), it is

probable that defenders perceiving a strong attacker, as indicated by more vigorous shell-rapping, may be catabolising Hc as an emergency energy reserve. However, Hc is more complex than glucose and glycogen, and takes a longer time to break down, which may explain the non-significant trend for decreasing  $[Hc]_{HL}$  with contest duration and the total number of bouts in defenders (Figures 3.13 and 3.14).

The positive correlations between  $[Hc]_{HL}$  and fight vigour shown by unsuccessful attackers indicate that Hc allows them to persist for longer in shell fights. Unsuccessful attackers decide to terminate encounters without first evicting the defenders. These individuals thus indicate the attackers' true level of persistence as they decide to give up once they have reached their endurance threshold, whereas the outcome for successful attackers is influenced by the defenders' decision to terminate the fight. Thus, greater concentrations of Hc appear to allow the contestants to perform a greater degree of activity before the onset of anaerobia and exhaustion, triggering the giving up decision.

High aerobic capacity has already been suggested to be advantageous to shell fighting hermit crabs in Chapter 2 (Mowles et al. 2008), although the modulation of Hc oxygen affinity by  $Ca^{2+}$  and  $Mg^{2+}$  appears to be of more benefit to attackers than to defenders. However, the present study suggests that the  $[Hc]_{HL}$  seems to be more important for defenders than attackers (Figures 3.3, 3.4 and 3.6). Perhaps attackers have a greater degree of access to metal ions from the aqueous environment with which to enhance their aerobic capacity, whereas this is restricted for defenders, which remain tightly withdrawn inside their shells, presumably relying on metal ions liberated from their exoskeletons (Henry et al. 1981), and greater concentrations of Hc to allow them to persist during aggressive encounters. Thus, it appears that the two different roles may adopt different strategies for enhancing aerobic capacity during shell fights.

The synthesis of Hc is a costly process, resulting in optimum concentrations being approached under different oxygen regimes (Spicer & Baden 2001). As not all individuals would possess the capacity to synthesise high levels of Hc, its concentration may reflect the physiological quality of the individual. Those with more Hc would therefore be better quality individuals, and the greater concentrations of Hc would make them better competitors, able to sustain vigorous activity for longer. This process would be analogous to the enhanced performance of athletes who have increased haemoglobin concentrations following altitude training (Adams et al. 1975; Heinicke et al. 2005). However, in addition to production costs, there may also be inherent disadvantages to an individual of maintaining a high level of haemocyanin, including potential oxidative damage and osmotic constraints imposed by

altering the composition of the haemolymph. Thus, the heightened  $[Hc]_{HL}$  may be beneficial to fighting hermit crabs; but may also be disadvantageous in other contexts, perhaps resulting in an optimal concentration, which would be seldom exceeded (A. Zahavi, pers. comm.).

The shell-rapping performed by attacking hermit crabs is assumed to be an example of a costly repeated signal, which communicates the stamina of the attacking crab as it is aerobically challenging to perform. The present data support this possibility as greater  $[Hc]_{HL}$  is beneficial to fighting hermit crabs, and this may be because it provides a greater scope for aerobic activity before the onset of anaerobic respiration, thus enhancing stamina. According to models of fighting where giving up is based on an individual cost threshold (i.e. the CAM, and in particular the E-WOA) repeated 'signals' challenge the performer's 'stamina' or maximum endurance capacity. As elements of these models appear to fit contest behaviour across a range of taxa, from hermit crabs (Briffa & Elwood 2000b) to red deer (Clutton-Brock & Albon 1979), it is likely that aerobic capacity via respiratory pigment concentration may be an important governing factor in many of these contests. The present data show that  $[Hc]_{HL}$  is important during hermit crab shell fights, and those possessing greater  $[Hc]_{HL}$  are more likely to win aggressive encounters. Thus, greater levels of respiratory pigment appear to enhance agonistic performance via their influence on aerobic capacity, and perhaps, therefore, stamina.



## **CHAPTER 4**

**Whole-organism performance capacity predicts resource holding potential in the hermit crab *Pagurus bernhardus*.**

### ***Abstract***

Animal contests often involve the production of repeated signals, which are assumed to be costly to perform, thereby demonstrating the contestants' ability to meet the physiological demands of engaging in aggressive encounters. A recent trend has been to test the costs of such signals by analysing fights with respect to proximate physiological consequences of aggressive behaviour such as energetic costs and endocrine changes. However, multiple physiological and morphological factors presumably combine to determine the overall performance capacity of an animal. Thus, physical fitness may be related to Darwinian fitness by constraining the strength of signal expression. This study examined the vigour and outcomes of hermit crab shell fights, after which locomotor capacities were measured in order to investigate the stamina of the contestants. Locomotor capacity was measured as the time taken to complete a 15 m distance along a circular water-filled raceway, from which mean and maximum speeds were calculated for each individual. Contest winners had faster mean speeds, and faster maximum speeds than losers. Thus, whole-organism performance capacities appear to be correlated with success in aggressive encounters.

## INTRODUCTION

Animal contests often involve the use of repeated signals which are assumed by theoretical models such as the cumulative assessment model (CAM: Payne 1998) and the energetic war of attrition (E-WOA: Payne & Pagel 1996; Payne & Pagel 1997) to be related to the resource holding potential (RHP) of the sender. In contrast to conventional cues such as body size (Caldwell & Dingle 1979; Riechert 1978), weapon size (Barki et al. 1997; Sneddon et al. 1997), and ornamentation (Lailvaux et al. 2005; Vanhooydonck et al. 2005), repeated signals are assumed to be related to the stamina of the sender, and in order to do this they are expected to be energetically demanding to perform. This is supported by empirical studies that show that the rate at which agonistic behaviours are performed may be linked to endurance. Furthermore, high display rates can enhance agonistic success over a wide range of taxa including hermit crabs (Briffa et al. 1998); fish (see Abrahams et al. 2005); toads (Sullivan & Walsberg 1985); lizards (Molina-Borja et al. 1998), birds (Weary et al. 1991) and red deer (Clutton-Brock & Albon 1979).

Many studies analyse fights with respect to their physiological consequences in order to quantify the proximate costs of agonistic behaviours such as signalling. These costs may accrue due to endocrine changes (Briffa & Elwood 2007; Sneddon et al. 2000b) and energetic consequences of fighting (Briffa & Elwood 2001; Briffa & Elwood 2002; Briffa & Elwood 2004; Neat et al. 1998; Schuett & Grober 2000; Sneddon et al. 1999; Thorpe et al. 1995). However, many interacting proximate factors are likely to influence contest behaviour. These include morphological differences in factors such as body size (Caldwell & Dingle 1979; Riechert 1978) and weapon size (Barki et al. 1997; Sneddon et al. 1997) in addition to physiological constraints. Ultimately it may be a combination of these factors that determines fighting ability. For example, in hermit crabs, increased concentrations of metal ions can influence fighting ability by modulating haemocyanin-oxygen binding (Mowles et al. 2008, Chapter 2), and heightened haemocyanin concentrations influences fighting ability similarly by increasing aerobic capacity and thereby stamina (Chapter 3). As such correlates of stamina are known to influence contest behaviour (e.g. Briffa & Elwood 2001; Mowles et al. 2008, Chapter 2, Chapter 3), and as repeated signals are assumed to demonstrate stamina, it would follow that stamina itself is likely to influence fighting ability.

Experiments into 'performance capacities' or 'whole-organism performance' (Huyghe et al. 2005; Perry et al. 2004) allow the quantification of the amount of activity that an organism is capable of undertaking, irrespective of the actual mechanisms involved. Such an approach

can thus investigate 'stamina' directly, avoiding the possibility that important effects could be missed if a single physiological or morphological system is focussed on. Performance capacities can be quantified as maximal rates of performance (the maximum speed at which an individual can perform a behaviour) or as maximal exertion (before the onset of exhaustion) (see Lailvaux et al. 2005), giving a measure of stamina (the capacity to perform a behaviour at a specific rate over a prolonged period). These measures reveal the ability of individuals to perform 'ecologically relevant tasks' such as running, jumping and biting, which have the potential to influence contests (Lailvaux & Irschick 2007). Such capacities should be the primary targets of selection (Lailvaux et al. 2004), with changes in the associated morphology and physiology occurring secondarily (Husak et al. 2007).

Animals studied in performance capacity experiments include various lizard species, e.g. *Anolis sp.* (e.g. Perry et al. 2004); *Lacerta sp.* (e.g. Clobert et al. 2000) and *Urosaurus sp.* (Robson & Miles 2000), as well as *Euoniticellus intermedius* dung beetles (Lailvaux et al. 2005). Such studies typically involve exercising animals until exhaustion on motorised treadmills (Brandt 2003; Sinervo et al. 2000) or around circular raceways (Lailvaux et al. 2005; Leal 1999). Strength is usually recorded by using force transducers in order to measure bite force in lizards (e.g. Huyghe et al. 2005) or pinch force in crabs (Sneddon et al. 2000a).

Many previous studies have found relationships between performance capacities and fighting ability, although the important aspects of performance capacity differ between species. For example, stamina was higher in the winners of agonistic encounters in *Uta stansburiana* (Brandt 2003) and *Anolis cristatellus* (Perry et al. 2004), and acceleration and jumping velocity are greater in winners of fights in *Anolis carolinensis* (Lailvaux et al. 2004), but see Huyghe et al. (2005). Further, the bite force of lizards (*G. galloti*, Huyghe et al. 2005; *A. carolinensis*, Lailvaux et al. 2004) and pinch force of crabs (*Carcinus maenas*, Sneddon et al. 2000a) are greater in the winners of fights.

As these examples show, many aspects of performance capacity are correlated with fighting ability. Another factor that may influence performance capacity and fighting ability is variation in morphology. For example, due to the mechanics of weaponry, differences in the morphology of jaws and claws will affect the strength of biting in lizards and pinching in crabs. Lizards that were successful in aggressive encounters have been shown to have relatively larger head sizes (Herrel et al. 1999; Huyghe et al. 2005), supporting the musculature necessary for providing a stronger bite (Perry et al. 2004). Similarly, the winners of shore crab fights have been demonstrated to have greater claw height and length to the dactyl irrespective of claw size. This would result in a greater pinching force by increasing

the mechanical leverage of the claw. As these particular claw dimensions would also present an increased surface area, this raises the possibility they may act as visual cues signalling the strength of competing crabs (Sneddon et al. 2000a). Similar cues may also be used in other species where the size of body parts correlates with strength, for example head size predicting bite force in lizards (Perry et al. 2004).

Several studies have found relationships between signal expression and performance capacities (e.g. Brandt 2003; Leal 1999). However, some display behaviours correlate with aspects of endurance while others do not. This difference may reflect the different aspects of performance that are important in agonistic behaviours in different species (see Huyghe et al. 2005). For example, the push-up displays performed by several lizard species were found to be correlated with endurance in *Anolis cristatellus* (Leal 1999), but only a non-significant trend was found in *Uta stansburiana* (Brandt 2003). The endurance of *U. stansburiana*, however, was found to be positively related to the duration of the compression display this species performs, possibly because the constraints placed on aerobic metabolism by maintaining the compressed body posture are more costly than performing push-ups (Brandt 2003).

Ornament size may also reflect performance capacities, for example relative dewlap size has been found to be an indicator of bite force in territorial, but not non-territorial *Anolis* species (Lailvaux & Irschick 2007; Vanhooydonck et al. 2005). Similarly, in a study focussing on the dung beetle *Euoniticellus intermedius*, Lailvaux et al. (2005) found that horn size was correlated with both strength and stamina. Thus, such signals may communicate performance capacity, and therefore fighting ability, to opponents (Lailvaux & Irschick 2007 and references therein).

If agonistic signals advertise stamina, they are likely to be costly to perform. This has been demonstrated in a range of studies (Briffa & Elwood 2001; Briffa & Elwood 2002; Briffa & Elwood 2004; Neat et al. 1998; Schuett & Grober 2000; Sneddon et al. 1999; Thorpe et al. 1995) and such costs may ensure signal honesty (Grafen 1990; Zahavi 1977). However, few studies have attempted to test directly the possibility that repeated signals correlate with ‘whole body’ performance capacities. This would be expected if these signals do in fact demonstrate the sender’s stamina.

In hermit crabs, fights commonly occur over the ownership of gastropod shells, with the opponents adopting two very different roles during these encounters. The ‘attacker’ (initiating crab) attempts to take the shell of the ‘defender’ (non-initiator), following an assessment period consisting of a series of bouts of ‘shell-rapping’, during which the defender remains

tightly withdrawn inside its shell. The attacker grips the shell of the defender closely with its walking legs, and begins to bring its own shell repeatedly into contact with that of the defender as a bout of raps in quick succession. A series of such bouts, separated by distinct pauses, is performed during the shell fight. Shell fights can end in one of two ways, either the defender decides to give up, allowing itself to be evicted by the attacker, which removes the defender through the aperture of the shell or the attacker may decide to give up first without having evicted the defender.

The vigour of shell-rapping is a key determinant of the outcome of shell fights, and it has been consistently shown that attackers that perform more raps per bout, leave shorter pauses between bouts of rapping (Briffa et al. 1998) and perform more powerful raps are more likely to win shell fights (Briffa & Elwood 2002). This suggests that the pattern of rapping contains information about fighting ability, acting as an honest signal (Briffa 2006) advertising the attacker's stamina (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998). The honesty of the signal appears to be maintained by the energetic costs incurred during its performance (Briffa 2006). Thus, the metabolic consequences of performing demanding signals may impose physiological limitations to behaviour, thereby precluding an animal from signalling beyond its capacity (Hurd 1997). Furthermore, the performance capacities of the crabs adopting each role may influence their fighting ability through their stamina. The shell-rapping signal presumably communicates the stamina of the attacker (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998), while the defender must persist in maintaining a grip inside its shell for the duration of the interaction. The aim of this study was to examine how performance capacities influence contest behaviour, in terms of the outcomes and vigour of contests using hermit crab shell fights as a model system. It was predicted that individuals that had greater performance capacities in terms of mean speed (stamina or maximum exertion) and maximum speed (maximum effort) would be better competitors in shell fights.

## MATERIALS AND METHODS

### *Study organisms*

Specimens of the common European hermit crab *Pagurus bernhardus* were collected between May 2007 and July 2007 from rock pools at Hannafore Point, UK (50° 20' N, 4° 27' W). The hermit crabs were kept in groups of 80-180 in 65 cm x 50 cm x 30 cm plastic tanks, filled with aerated seawater to a depth of 15 cm (~50 l). These were maintained at 15°C in a temperature controlled laboratory and were fed on a diet of catfish pellets.

### *Staging fights and recording behaviour*

Hermit crabs were removed from their gastropod shells by cracking the shells open in a bench vice. They were then sexed; only male crabs were used in order to avoid sex-related behavioural differences as found in previous studies (Neil & Elwood 1985). Study animals were examined for physical damage, and only crabs that were (i) free from obvious parasites, (ii) undamaged and (iii) intermoult were used in the experimental trials. Unused crabs were provided with new shells and were returned to the site from which they were originally collected within a fortnight of collection.

The study crabs were assigned to pairs consisting of a small crab and a large crab (mean weights  $\pm$  s.e.; small,  $0.802 \pm 0.016$  g; large,  $1.053 \pm 0.023$  g). The larger crab of the pair (potential attacker) was provided with a *Littorina littorea* shell that was only 50% of its preferred shell weight, while the smaller crab (potential defender) was provided with a *L. littorea* shell which would be 100% adequate for the larger crab of the pair. The preferred shell size was determined using a regression that relates crab weight to preferred shell weight (Briffa & Elwood 2007).

Following shell allocation, crabs were isolated in 95 mm diameter crystallizing dishes filled with aerated seawater, and allowed to acclimate for 16 hours prior to being used in fights. Encounters were staged in two groups to which the pairs of crabs were randomly allocated. In the control group, ( $N = 11$  pairs) the fight was terminated as the attacker made initial contact with the shell of the defender. This process ensured that control crabs were healthy individuals that were prepared to fight, but did not fight. The second group ( $N = 67$  pairs) contained fights that were allowed to proceed until their conclusion, when either the

defender allowed itself to be evicted from its shell ( $N = 46$  pairs); or the attacker terminated the fight without first having evicted the defender ( $N = 21$  pairs).

Fights were staged between 0930 and 1200 hours each day. A large crab was placed in an arena consisting of a 95 mm diameter crystallizing dish, filled to a depth of 4 cm with aerated seawater, divided in half by a piece of transparent acrylic and containing a 1 cm thick sandstone tile as a base. The dish was then placed in an observational chamber, behind a two-way mirror, so that the crabs could not see the observer. The small crab was then introduced into the other half of the arena containing the large crab, and allowed to acclimate for 10 seconds before the transparent partitioning was removed. This ensured that both crabs were aware of each other before being allowed physically to interact. Once the partition was removed, the ensuing behaviours and pattern of shell rapping was recorded using a Psion Workabout MX (Psion Teklogix Inc., Mississauga, ON, Canada) configured as a time-event recorder using The Observer 5.0 (Noldus IT, Wageningen, The Netherlands) event-recording software. The pattern of shell-rapping (contest duration in seconds, total number of raps, total number of bouts of rapping, mean number of raps/bout and mean pause duration in seconds) was then analysed in order to calculate the vigour of shell rapping performed by attackers during each fight (see ethogram in Chapter 2 Materials and Methods). Following shell fights, the crabs were fed, and kept individually for a 45 hour recovery period before being used in the performance capacity trials.

### ***Performance capacities***

The hermit crabs were removed from their shells by means of a bench vice. However, this time they were provided with 25% adequate shells made up to 100% preferred shell weight by the use of a plasticine coating. This ensured that the crabs were unable to retract into their shells when stimulated to run, yet were carrying a shell of optimal weight for their body size. They were allowed a 2 hour acclimation period to adjust to these new shells before being used in the performance capacity trials, which took place between 1230 and 1500 hours each day.

Following the 2 hour acclimation period, each crab was placed individually in a circular raceway 90 cm in circumference, with a 6 cm track-width filled to a depth of 5 cm with aerated seawater. Each crab was stimulated to run by a series of light taps on its shell using a plastic probe. Once in motion, the crab was pursued at its own pace by the probe, which was



kept one body length behind the running crab. The crab was encouraged to move non-stop around the raceway by receiving a gentle tap every time it stopped moving, as intermittent locomotion in crustaceans has been shown to increase work capacity (Weinstein & Full 2000). The time it took to complete each lap was recorded for 16.75 laps (15 m), as well as the mean speed ( $\text{ms}^{-1}$  over 15 m) and maximum speed ( $\text{ms}^{-1}$  for fastest lap). The time taken to reach the distance of 15 m was recorded rather than the time until exhaustion as the hermit crabs were difficult to fatigue. Similar results have been gained for other crabs (40–45 minutes of walking for *Carcinus maenas*, Sneddon et al. 1999). The 15 m distance resulted in a drop in speed to a stable level, and took approximately 10 minutes to perform, allowing the handling of adequate sample sizes (see Perry et al. 2004). After the performance capacity trials, the crabs were immediately used for muscle analysis (see Chapter 5).

## RESULTS

### FIGHTING AND PERFORMANCE

As the crabs had been provided with food and a 45 hour recuperation period after fighting, the locomotor capacities were not predicted to have been depleted as a result of having been in a fight; however this was tested using a repeated measures ANOVA. The between subject factor in the model was fight status (fought or the unfought control group), while the repeated measure was either the mean speed ( $\text{ms}^{-1}$ ) or the maximum speed ( $\text{ms}^{-1}$ ) for each role over the 15 m distance. The ANOVA results are reported with their respective effect size estimates ( $\eta^2$ , 'eta squared').

There was no significant difference between the mean speed of the crabs and their fight status ( $F_{1,75} = 0.529$ ,  $P = 0.4694$ ), and no significant interaction between the role adopted by the crabs and whether they fought ( $F_{1,75} = 0.006$ ,  $P = 0.9391$ ). Similarly, the maximum speed of the crabs did not change with fighting ( $F_{1,75} = 0.167$ ,  $P = 0.6840$ ), and no significant interaction was found between the role of each crab and their fight status ( $F_{1,75} = 0.335$ ,  $P = 0.5647$ ).

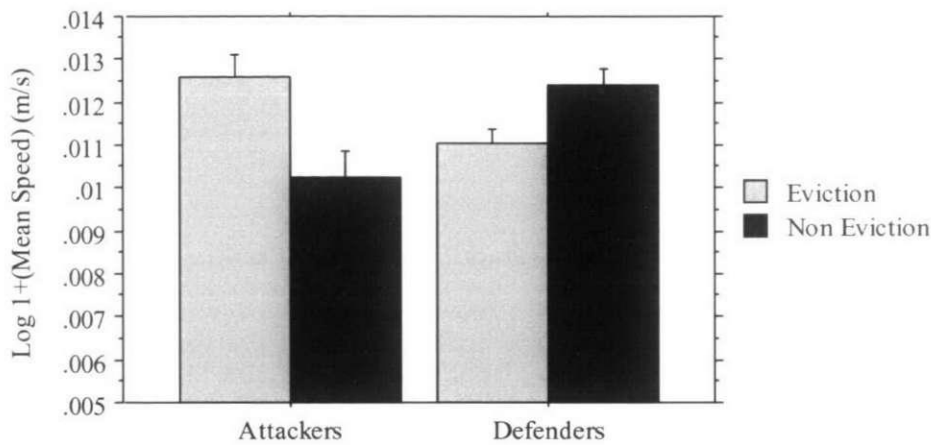
### FIGHT OUTCOMES AND PERFORMANCE CAPACITIES

#### *Repeated Measures ANOVA – Differences in mean speed between roles and outcomes*

As participating in a fight did not appear to affect the locomotor capacities of the hermit crabs, the control group was excluded from further analysis. The following analyses thus focus on the differences between fight outcomes. The effects of fight outcome on locomotor capacity were determined by performing a repeated measures ANOVA. The between subject factor in the model was outcome (eviction or non-eviction), while the repeated measure was either the mean speed, or maximum speed ( $\text{ms}^{-1}$ ) of each role (attacker or defender).

There was no difference in the mean speed between the outcomes ( $F_{1,64} = 0.989$ ,  $P = 0.3238$ ), or between the roles adopted by the crabs ( $F_{1,64} = 0.302$ ,  $P = 0.5843$ ). However, a significant interaction was found between role and outcome ( $F_{1,64} = 13.266$ ,  $P = 0.0005$ ,  $\eta^2 =$

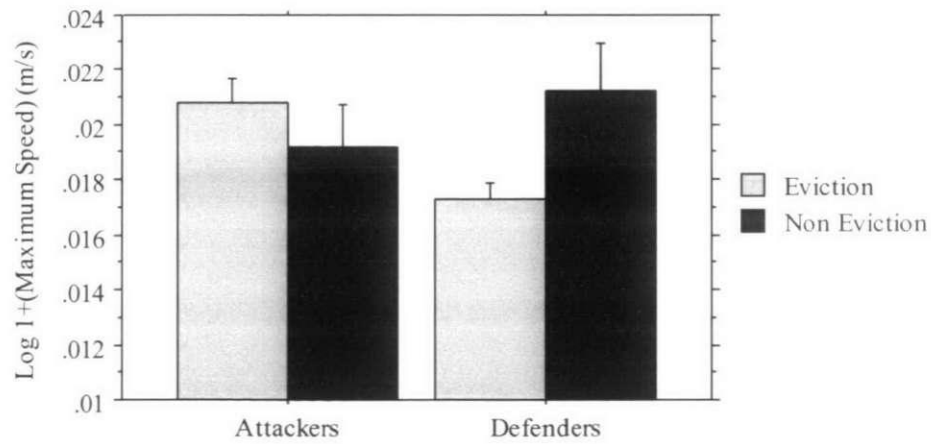
0.092; Figure 4.1), where the mean speed was higher in winners than losers(i.e. attackers that evicted defenders and defenders that resisted eviction).



**Figure 4.1.** Mean speed of each role in relation to the outcome of the fight.

***Repeated Measures ANOVA – Differences in maximum speed between roles and outcomes***

There was no difference in the maximum speed between the fight outcomes ( $F_{1,64} = 0.843, P = 0.3621$ ), or between the role adopted by the crabs  $F_{1,64} = 0.651, P = 0.4228$ . However, there was a significant interaction between role and outcome ( $F_{1,64} = 8.693, P = 0.0045; \eta^2 = 0.041$ ; Figure 4.2) where the maximum speed was higher in winners than losers (i.e. attackers that evicted defenders and defenders that resisted eviction).



**Figure 4.2.** Maximum speed of each role in relation to the outcome of the fight.

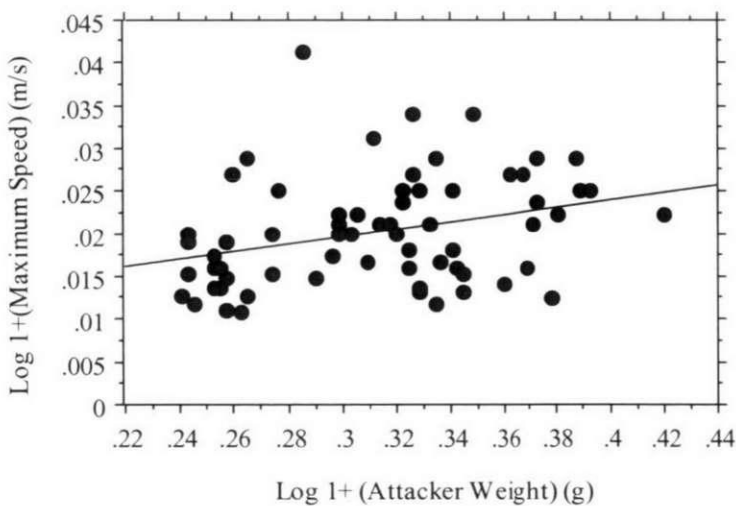
*ANCOVA – Performance Capacities of Attackers only*

Interaction effects may mask differences between outcomes within each role. As repeated measures ANOVAs do not allow post-hoc comparisons between specific groups, one-factor ANCOVAs were used to examine each role independently. The dependent variable in these models was either the mean speed ( $\text{ms}^{-1}$ ) or maximum speed ( $\text{ms}^{-1}$ ) of the competitors. The factor included in each model was fight outcome (eviction or non-eviction), and the weight of the crabs was included as a covariate as size may influence performance of the locomotion task.

When the mean speeds of attackers were analysed independently of defenders, no significant interaction effects involving the covariate were detected, and were thus deleted from the model. In the recalculated model, attackers that effected evictions had faster mean speeds than attackers that did not evict defenders ( $F_{1,63} = 4.316$ ,  $P = 0.0418$ ; Figure 4.1). There was no relationship between mean speed and the size of the attackers ( $F_{1,63} = 1.914$ ,  $P = 0.1715$ ).

When the maximum speeds of attackers were analysed on their own, no significant interaction effects involving the covariate were detected, and were thus deleted from the model. There was no difference in the maximum speeds between outcomes ( $F_{1,63} = 0.001$ ,  $P = 0.9785$ ; Figure 4.2). However, there was a significant positive relationship between the maximum speed and the size of the attackers ( $F_{1,63} = 5.784$ ,  $P = 0.0191$ ; Figure 4.3).

A positive correlation was also found between the mean and maximum speeds performed by attackers ( $Z = 8.014$ ,  $P < 0.0001$ ).



**Figure 4.3.** Maximum speed in relation to attacker weight.

### ***ANCOVA – Performance Capacities of Defenders only***

Again, in order to investigate defenders independently of attackers, one-factor ANCOVAs were performed. The dependent variable in these models was either the mean speed ( $\text{ms}^{-1}$ ) or maximum speed ( $\text{ms}^{-1}$ ) of the competitors. The factor included in each model was fight outcome (eviction or non-eviction), and the weight of the crabs was included as a covariate.

In the analysis of mean speed, there was no significant interaction effect involving the covariate, and this was therefore deleted from the model. In the recalculated model, defenders that resisted eviction achieved higher mean speeds than those that were evicted ( $F_{1,63} = 5.200$ ,  $P = 0.0259$ ; Figure 4.1). There was no relationship between the mean speed and the size of the defenders ( $F_{1,63} = 0.898$ ,  $P = 0.3470$ ).

In the analysis of maximum speed, there was no significant interaction effect involving the covariate, and this was therefore deleted from the model. In the recalculated model, defenders that retained their shells had faster maximum speeds than unsuccessful defenders that were evicted ( $F_{1,63} = 5.412$ ,  $P = 0.0232$ ; Figure 4.2). There was no relationship between the maximum speed and the size of the defenders ( $F_{1,64} = 1.495$ ,  $P = 0.2260$ ).

Similarly to attackers, positive correlation was also found between the mean and maximum speeds performed by defenders ( $Z = 4.345$ ,  $P < 0.0001$ ).

### **CORRELATIONS BETWEEN FIGHT VIGOUR AND PERFORMANCE CAPACITIES**

In order to determine how locomotor capacities may influence fight vigour, a series of one-factor ANCOVAs was performed, which included either the mean speed or maximum speed as the dependent variable, and fight variables associated with vigour as covariates (see ethogram in Chapter 2 Materials and Methods). Fight outcome was included as a factor in the model as it has been shown in the previous section to be influenced by the locomotor capacities of the contestants. However, in order to investigate possible relationships between performance capacities and contest duration, only losing individuals were analysed (attackers that did not effect evictions and defenders that were evicted) as these individuals that give up are those that provide information on persistence. As these analyses did not involve a factor,

they were analysed using correlation Z-tests instead of ANCOVAs. Bivariate scattergrams with regression lines have been presented in order to illustrate the direction of the effects.

### ***Performance Capacities in Attackers***

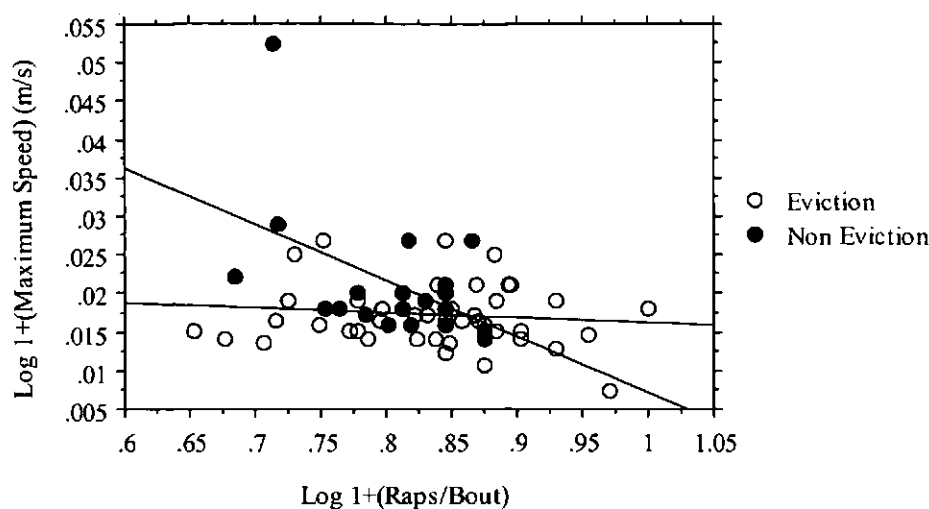
In attackers there were no significant interaction effects involving the covariate, and these were therefore deleted from the models. In the recalculated models, there were no relationships between mean speed and total raps ( $F_{1,61} = 0.004$ ,  $P = 0.9475$ ), total bouts ( $F_{1,61} = 0.026$ ,  $P = 0.8723$ ), raps/bout ( $F_{1,61} = 0.534$ ,  $P = 0.4676$ ), or mean pause duration ( $F_{1,58} = 0.501$ ,  $P = 0.4818$ ). There was also no correlation between mean speed and contest duration ( $Z = 0.379$ ,  $P = 0.7048$ ).

There were no significant relationships between the maximum speed of attackers and the contest duration ( $F_{1,61} = 0.299$ ,  $P = 0.5863$ ), total raps ( $F_{1,61} = 0.093$ ,  $P = 0.7614$ ), total bouts ( $F_{1,61} = 0.089$ ,  $P = 0.7670$ ), raps/bout ( $F_{1,61} = 0.179$ ,  $P = 0.6737$ ), or mean pause duration ( $F_{1,61} = 0.238$ ,  $P = 0.6272$ ). There was also no correlation between mean speed and contest duration ( $Z = 0.597$ ,  $P = 0.5507$ ).

### ***Performance Capacities in Defenders***

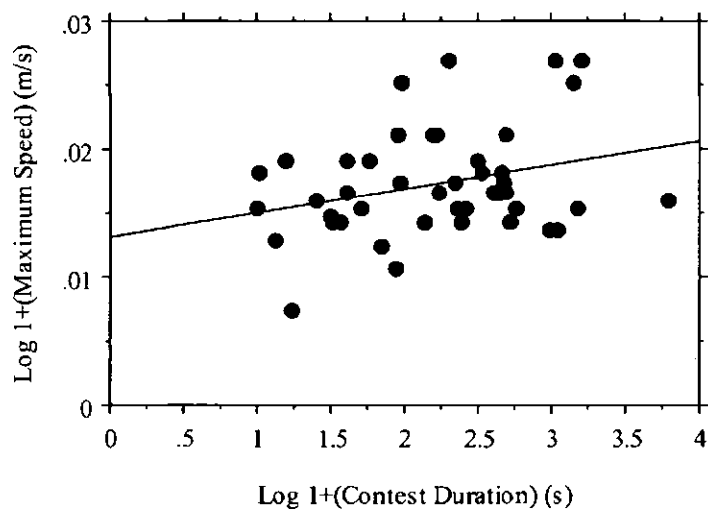
In defenders, there were no significant interaction effects involving the covariate, and these were deleted from the models. In the recalculated models, there were no relationships between mean speed and total raps ( $F_{1,62} = 0.621$ ,  $P = 0.4338$ ), total bouts ( $F_{1,62} = 0.922$ ,  $P = 0.3407$ ), raps/bout ( $F_{1,62} = 2.395$ ,  $P = 0.1268$ ), or mean pause duration ( $F_{1,62} = 1.890$ ,  $P = 0.1744$ ). There was also no correlation between mean speed and contest duration ( $Z = 1.273$ ,  $P = 0.2030$ ).

There were no significant effects of the maximum speed of defenders on the total number of raps ( $F_{1,62} = 2.344$ ,  $P = 0.1308$ ), or total number of bouts of rapping ( $F_{1,62} = 2.846$ ,  $P = 0.0966$ ) in the contest. However, there was a negative relationship between the maximum speed of defenders and the number of raps/bout ( $F_{1,61} = 10.102$ ,  $P = 0.0023$ ). There was also an interaction ( $F_{1,61} = 7.289$ ,  $P = 0.0090$ ; Figure 4.4) between the outcome of the contest and the number of raps/bout. In defenders that were evicted, there was no relationship between the maximum speed and the number of raps/bout ( $R^2 = 0.012$ ,  $F_{1,42} = 0.489$ ,  $P = 0.4880$ ), whereas in defenders that retained their shells maximum speed declined with raps/bout ( $R^2 = 0.234$ ,  $F_{1,19} = 5.808$ ,  $P = 0.0263$ ).



**Figure 4.4.** Maximum speed of defenders in relation to the number of raps/bout and the outcome of the contest.

However, these relationships appear to be driven by an outlier where one individual had an unusually high maximum speed (Grubbs test:  $Z = 5.6$ ,  $P < 0.05$ ). Its elimination removes the significance of the result ( $F_{1,61} = 1.451$ ,  $P = 0.2330$ ) for the effect of maximum speed on raps/bout, with no significant interaction effects. There was no significant effect between the maximum speed of defenders and the mean pause duration ( $F_{1,59} = 1.597$ ,  $P = 0.2112$ ). However, in defenders there was a non-significant positive correlation between maximum speed and contest duration ( $Z = 1.957$ ,  $P = 0.0503$ ; Figure 4.5).



**Figure 4.5.** Maximum speed of defenders in relation to contest duration.

## DISCUSSION

The winners of hermit crab shell fights had higher performance capacities than losers, in terms of both mean speeds and maximum speeds attained on the circular raceway. The aspects of performance capacity that were important appeared to differ between the roles adopted by the crabs. Mean speed appears to be important to competitive success in both attackers and defenders as successful individuals (attackers that effect evictions and defenders that resist eviction) had higher mean speeds than unsuccessful individuals (attackers that failed to effect evictions, and defenders that were evicted; Figure 4.1). Maximum speed, however, appears to be a better indicator of performance for defenders than attackers. Although the interaction shows that successful individuals attain higher maximum speeds than unsuccessful individuals (Figure 4.2), there is no significant difference between the maximum speeds of attackers that won (those that effected evictions) and attackers that lost (those that failed to evict defenders). However, successful defenders that retained their shells attained higher maximum speeds than unsuccessful defenders that lost their shells, indicating that maximum speeds, and their associated mechanisms are more important to the competitive success of defenders than attackers. A significant positive relationship was detected between the maximum speed of attackers and attacker weight (Figure 4.3). This trend is likely to be a function of size as larger individuals would have larger leg lengths and be able to cover more ground in a single step. The relationship was not present in defenders, which were smaller than attackers, and have a slightly smaller size range. Thus, there may be insufficient resolution to generate a similar trend in defenders. A non-significant trend was also found between the maximum speed of defenders and the duration of the contest (Figure 4.5), suggesting that defenders with higher maximum speeds may be able to resist eviction for longer than those with lower maximum speeds.

During hermit crab shell fights, the crabs adopt two distinct roles: attacker and defender, and each role performs very different activities during the fight. The attacking crab performs the most obviously demanding behaviour by engaging in shell-rapping. It has been suggested that this repeated signal demonstrates the attacker's stamina, and the vigour of its performance is related to the metabolic and physiological correlates of stamina such as elevated *L*-lactate (Briffa & Elwood 2001; Briffa & Elwood 2004; Briffa & Elwood 2005) and haemocyanin concentration (Chapter 3). Analysis of the vigour of shell rapping has demonstrated that attackers that rap more vigorously are more likely to evict defenders,



performing more raps per bout, leaving shorter pauses between bouts of rapping (Briffa et al. 1998), and performing more powerful raps (Briffa & Elwood 2002).

As attackers that are able to withstand or avoid the costs associated with shell-rapping would be able perform the signal more vigorously, such individuals with higher stamina (the capacity to perform a behaviour at a specific rate over a prolonged period) would be predicted to win more fights. Despite performing the less obviously demanding behaviour of the two roles during shell fights, stamina is likely to be important to defenders as well. These individuals remain tightly withdrawn inside their defended shells for the duration of the contest, and must resist the attacker's attempts at eviction. It is likely that the oxygen availability within the confines of the shell will fall during the fight, challenging the defender's aerobic metabolism and endurance. Indeed, defenders with higher concentrations of respiratory pigment are more likely to win such encounters (Chapter 3). In addition, the defenders must actively grip the contested shell in order to resist the attacker's attempts at eviction, and individuals with higher levels of stamina would be more likely to do this successfully. Thus, although the importance of specific pathways may differ, overall stamina is likely to be important to both roles during shell fights. This is supported by the interaction illustrated in Figure 4.1, where winners had higher mean speeds than losers. The analysis of each role independently confirmed this as attackers that won (those that evicted defenders) had higher mean speeds than attackers that lost (those that failed to effect evictions), and defenders that won (those that resisted eviction) had higher mean speeds than defenders that lost (those that were evicted).

Winners of shell fights also had higher maximum speeds on the circular raceway than losers (Figure 4.2). However, the independent analysis of each role revealed that there is only a significant difference between the maximum speeds of winners and losers in defenders. Successful defenders had higher maximum speeds than unsuccessful defenders, whereas there was no significant difference between the two outcomes for attackers. Furthermore, there was a non-significant trend for a positive relationship between maximum speed and contest duration in defenders but not in attackers, suggesting that the mechanisms associated with burst activity may be related to defender resistance.

It is not unexpected to find a difference such as this between the two roles. Attackers and defenders have already been shown to respond to different thresholds in terms of physiological costs, with giving up in attackers being associated with high *L*-lactate whereas giving up in defenders is associated with perceiving a strong attacker and failing to mobilise glucose (Briffa & Elwood 2001; Briffa & Elwood 2004; Briffa & Elwood 2005).

Furthermore, it appears that attackers may utilise metal ions to increase their aerobic capacity during fights (Mowles et al. 2008, Chapter 2) whilst defenders rely on increased haemocyanin concentrations (Chapter 3). Similarly, attackers and defenders may rely on different aspects of performance capacity during shell fights, and this may illustrate the difference between maximum endurance and maximum effort. Maximum endurance may be more important to attackers than maximum effort (i.e. stamina rather than burst activity). This is congruent with the E-WOA and CAM models, which predict that RHP is dependent on stamina. Unlike attackers, it appears that maximum effort is important to defenders in addition to endurance. Perhaps typically anaerobic burst activity allows defenders to hold fast onto the shell during critical moments such as bouts of rapping or periods where the attacker attempts to prise them out of it.

Different aspects of performance capacity have also been shown to be important within members of the same species in the lizard *Anolis carolinensis* (Lailvaux et al. 2004). Here there are two alternative male phenotypes that resolve contests using different methods. These correlate with different aspects of performance capacity, in this case strength or speed. ‘Heavyweight’ males are larger than lightweight males, and have relatively large heads for their size. The ‘heavyweight’ males rely on high bite performance to win fights, whereas ‘lightweight’ males utilise greater jumping velocities and acceleration in order to secure territories more quickly during disputes, thereby gaining ‘resident male advantage’ (Lailvaux et al. 2004). Similar differences in performance capacities and signal expression have been found between various *Anolis* species with differing levels of territoriality. Lailvaux et al. (2007) found that dewlap size did not predict bite force in non-territorial species of *Anolis*, but did in territorial species, as would be expected for individuals that aggressively defend territories. Thus, differences in behaviour and life histories would select for different aspects of performance that are relevant to contest behaviour (see Huyghe et al. 2005).

Analysing the performance capacities of animals allows the actual quantification of strength or stamina, rather than a proxy as provided by the analysis of physiological correlates of these variables (such as *L*-lactate, glucose, glycogen and respiratory pigments for example). This holistic approach takes into account all of the proximate mechanisms that may influence the stamina of the study organism rather than extracting single variables that may be correlated with fighting ability. Thus, performance capacities may be more relevant to the outcome of contests than any physiological or morphological component in isolation. This is supported by the effect size estimates for the main results found in this study in comparison to those found in the haemocyanin experiment (Chapter 3). Here, the  $\eta^2$  for the

interaction in mean speed between fight outcomes in each role (winners had higher mean speeds than losers; Figure 4.1) was  $\eta^2 = 0.092$ , and the  $\eta^2$  for the interaction in maximum speed between fight outcomes in each role (winners had higher mean speeds than losers; Figure 4.2) was  $\eta^2 = 0.041$ . However, the  $\eta^2$  for the interaction in  $[\text{Hc}]_{\text{HL}}$  between fight outcomes in each role (winners had higher  $[\text{Hc}]_{\text{HL}}$  than losers; Figure 3.3) was  $\eta^2 = 0.02$ , and the  $\eta^2$  for the interaction in haemocyanin residuals between fight outcomes in each role (winners had higher  $[\text{Hc}]_{\text{HL}}$  than losers; Figure 3.4) was also  $\eta^2 = 0.02$ . Thus, the performance capacities had stronger effect size estimates than  $[\text{Hc}]_{\text{HL}}$  alone. Although it is likely that  $[\text{Hc}]_{\text{HL}}$  would contribute to performance capacity by influencing aerobic potential, many other factors are not taken into account by analyzing a specific factor such as this. Furthermore, the stronger effect size estimates generated by the whole-organism approach reveal that performance capacities are a better predictor of fighting ability than  $[\text{Hc}]_{\text{HL}}$  alone.

Studies examining the performance capacities of invertebrates are rare, with the two primary examples being the strength and stamina of the dung beetle *Euoniticellus intermedius* in relation to signal expression (Lailvaux et al. 2005) and the strength of the shore crab *Carcinus maenas* in relation to fighting ability (Sneddon et al. 2000a). Although previous studies have exercised shore crabs to exhaustion (Sneddon et al. 1999; Sneddon et al. 2000b), this has been done in order to measure the physiological consequences of exertion, rather than as an attempt to quantify stamina or relating it to the outcomes of encounters. Similarly, terrestrial crustaceans (ghost crabs, *Oxypode quadrata*) have been used to study the energetics of locomotion (for a review see Weinstein & Full 2000), but not to study performance capacities. The present study is thus the first to attempt to quantify the stamina of a crustacean directly by using performance capacities, and the first to relate it to fighting ability in an invertebrate.

Theoretical models such as the CAM (Payne 1998) and the E-WOA (Payne & Pagel 1996; Payne & Pagel 1997), although assuming self-assessment, predict that repeated ‘signals’ reflect the stamina, and thereby RHP, of opponents during contests. This study supports these assumptions by demonstrating that stamina itself is related to success in hermit crab shell fights. However, despite the whole-organism approach resulting in stronger effect size estimates than previous work on certain proxies of stamina (Chapter 3), there are differences in the aspects of performance capacity that are important depending on the tactics employed during encounters. This highlights that different aspects of performance capacity are functionally important in different contexts, and perhaps more than one measure of performance capacity may be required in order to understand the mechanisms of fighting

ability. Furthermore, this is in concordance with previous studies that demonstrate that the proximate mechanisms dictating the giving up decision differ between these two roles. In the shell fights of hermit crabs, multiple physiological and morphological factors may contribute towards RHP, although it appears that the overall metrics of the opponents and their performance capacities may be better predictors of agonistic success than any one of these components in isolation.

## **CHAPTER 5**

### **Muscle quality and contest behaviour in the hermit crab**

*Pagurus bernhardus.*

## ***Abstract***

Animal contests often include the production of repeated signals which are assumed to advertise the stamina, and thereby the resource holding potential (RHP) of the sender. A recent trend has been to examine fighting ability with respect to the physiological correlates of stamina in order to test the idea that fighting is costly. However, discrete physiological factors can only act as proxies for stamina, and experiments that examine whole organism performance capacities are more likely to produce accurate estimates of how stamina may affect fighting ability. Despite this, there may be situations where ecologically relevant performance capacities cannot be recorded, and surrogate physiological measures must be used instead. This study examined the vigour and outcomes of hermit crab shell fights, after which the crabs' abdominal muscles were dissected out and analysed for relative size and protein content. These measurements provide a proxy for strength, which is otherwise impossible to obtain. There was no difference in muscle protein between winners and losers, although overall winners showed a non-significant trend for larger muscle/body size ratios, and successful attackers had significantly higher muscle/body weight ratios than unsuccessful attackers. Thus, muscle quality does not affect fighting ability, although muscle size does. It is likely that relatively larger muscles increase the strength of competitors, allowing attackers to perform harder shell raps, and defenders to hold on more strongly during aggressive encounters.

## INTRODUCTION

Contests in animals often involve the use of repeated signals which may demonstrate the resource holding potential (RHP) of the sender by advertising their stamina. This is a known correlate of agonistic success as illustrated by experiments into performance capacity (Chapter 4, Brandt 2003; Perry et al. 2004), which have empirically demonstrated that individuals of high stamina can perform agonistic signals at a greater rate, and are more likely to win aggressive encounters than individuals of lower stamina. If repeated signals advertise stamina they should be energetically demanding to perform. This is supported by empirical studies that have measured various physiological changes associated with agonistic behaviour (Briffa & Elwood 2001; Briffa & Elwood 2002; Briffa & Elwood 2004; Neat et al. 1998; Schuett & Grober 2000; Sneddon et al. 1999; Thorpe et al. 1995).

Another measurement of performance capacity that may be important to fighting animals is strength. For example, lizards with higher bite forces are more likely to win fights (Huyghe et al. 2005; Lailvaux et al. 2004) as are crabs with higher claw strength (Sneddon et al. 2000a). Indeed, strength may be visually assessed using indexes such as the size of body parts (Perry et al. 2004; Sneddon et al. 2000a) or ornaments (Lailvaux et al. 2005), or during agonistic displays where signals are performed that correlate with sender strength (Lailvaux & Irschick 2007; Vanhooydonck et al. 2005).

Studies that measure strength directly tend to quantify it by using force transducers, which can measure bite force in lizards (e.g. Huyghe et al. 2005) and pinch force in crabs (Sneddon et al. 2000a). Lailvaux et al. (2005), however, used a novel and simple method to quantify the strength of the dung beetle *Euoniticellus intermedius* by measuring the mass required to pull a beetle from an artificial burrow. Dung beetles engage in fights over burrows and the mating rights to females therein. This contest system sees males attempting to push others from the burrow, whilst defending their territory and mates (see Lailvaux et al. 2005 and references therein). This is analogous to other 'asymmetrical' contest systems where one opponent attempts to take a resource from another, such as contests over the guarded mates of rival males in species such as amphipods (Dick & Elwood 1996), damselflies (Cordero 1999) and newts (Verrell 1986); over occupied shelters in stomatopods (Shuster & Caldwell 1982); and over the ownership of occupied gastropod shells in hermit crabs (Elwood & Briffa 2001). It is likely that opponents in these cases may benefit from high levels of strength, in which case they would be able to put up a greater resistance, or greater pulling power, and would be more likely to win aggressive encounters.

In hermit crab shell fights, the opponents adopt two distinctly different roles during the encounter: attacker and defender. The attacking crab initiates the encounter and attempts to take the gastropod shell from the non-initiator, which attempts to defend its shell by withdrawing tightly inside it. The attacker then performs a repetitive signalling behaviour called 'shell-rapping'. The attacker grips the shell of the defender closely with its walking legs, and begins to bring its own shell repeatedly into contact with that of the defender as a bout of raps in quick succession. A series of such bouts, separated by distinct pauses are performed during the shell fight. Shell fights can end in one of two ways, either the defender decides to give up; allowing itself to be evicted by the attacker, which removes the defender through the aperture of the shell; or the attacker may decide to give up first without having evicted the defender.

The vigour of shell-rapping is a key determinant of the outcome of shell fights, and it has been consistently shown that attackers that perform more raps per bout, leave shorter pauses between bouts of rapping (Briffa et al. 1998), and perform more powerful raps are more likely to win shell fights (Briffa & Elwood 2002). This suggests that the pattern of rapping contains information about fighting ability, advertising the attacker's stamina (Briffa & Elwood 2000a; Briffa & Elwood 2000c; Briffa et al. 1998) and perhaps strength (Briffa & Elwood 2000c; Briffa & Elwood 2002). In the previous chapter (Chapter 4), stamina was demonstrated to be important to both attackers and defenders during shell fights. However, other aspects of performance capacity may also be important to fighting hermit crabs. For example, strength is likely to be important to crabs engaging in shell fights as it may influence the ability of defenders to hold fast onto their shells, as well as the ability of the attacker both to pull the defender from its shell, and to engage in shell rapping.

The strength of hermit crabs is difficult to measure, especially with respect to the muscle groups used in shell fighting. When shell rapping, attackers bring their own shell into contact with that of the defenders using a swinging motion where the crabs articulate at the abdomen. During this process, defenders hold onto their shell using modified uropods. Thus, both roles potentially rely on their abdominal musculature during these encounters. The ideal measurement would be to measure the force required to pull a hermit crab from its shell, following the method used for extracting dung beetles from their burrows in Lailvaux et al. (2005). However, hermit crabs defend their shells with great resistance, and any attempt to measure strength by using the force required to pull them out of the shell would likely lead to the inhumane death of the crabs. An alternative approach is to select a proxy of strength, similar to studies that have measured various physiological variables as proxies of stamina



(e.g. Briffa & Elwood 2001; Briffa & Elwood 2002; Briffa & Elwood 2004; Neat et al. 1998; Schuett & Grober 2000; Sneddon et al. 1999; Thorpe et al. 1995). A good proxy for strength would be the quality of the abdominal musculature, which can be analysed physiologically in terms of muscle protein concentration, or morphologically in terms of relative size (i.e. muscle/body weight ratios). Larger muscles are likely to be stronger, providing a greater contraction force. Similarly, muscle protein content is also likely to be related to muscle strength (see Lohuis et al. 2007). Muscles with higher protein contents may be of better quality as they would contain relatively more fibres devoted to contraction than other substances such as lipids and water.

The aim of this study was to examine how abdominal muscle condition was related to contest behaviour in hermit crabs, both in terms of fight vigour and fight outcomes. The abdominal muscle protein concentration and muscle/body weight ratios were calculated in order to take a reasonable surrogate measure of strength, and it was predicted that individuals with better quality muscles (i.e. higher concentrations of muscular protein and higher muscle/body weight ratios) would be better competitors in shell fights.

## MATERIALS AND METHODS

### *Study organisms*

Specimens of the common European hermit crab *Pagurus bernhardus* were collected between May 2007 and July 2007 from rock pools at Hannafore Point, UK (50° 20' N, 4° 27' W). The collected crabs were restricted to a size range sufficient for the extraction of adequate muscle samples for analysis (crab weight = 0.61 – 1.63 g).

The hermit crabs were kept in groups of 80-180 in 65 cm x 50 cm x 30 cm plastic tanks, filled with aerated seawater to a depth of 15 cm (~50 l). These were maintained at 15°C in a temperature controlled laboratory and were fed on a diet of catfish pellets.

### *Staging fights and recording behaviour*

Hermit crabs were removed from their gastropod shells by cracking the shells open in a bench vice. They were then sexed as only male crabs were used in order to avoid sex-related behavioural differences as found in previous studies (Neil & Elwood 1985). Study animals were examined for physical damage, and only crabs which were (i) free from obvious parasites, (ii) undamaged and (iii) intermoult were used in the experimental trials. Unused crabs were provided with new shells and were returned to the site from which they were originally collected within a fortnight of collection.

The study crabs were assigned to pairs consisting of a small crab and a large crab (mean weights  $\pm$  s.e.; small,  $0.802 \pm 0.016$  g; large,  $1.053 \pm 0.023$  g). The larger crab of the pair (potential attacker) was provided with a *Littorina littorea* shell that was only 50% of its preferred shell weight, while the smaller crab (potential defender) was provided with a *L. littorea* shell which would be 100% adequate for the larger crab of the pair. The preferred shell size was determined using a regression which related crab weight to preferred shell weight (Briffa & Elwood 2007).

These trials were carried out using the same shell fights as the previous performance capacity experiment, although the data are separated into the performance capacity and muscle quality chapters (Chapter 4 & 5) for clarity. Following shell allocation, crabs were isolated in 95 mm diameter crystallizing dishes filled with aerated seawater, and allowed to acclimate for 16 hours prior to being used in fights. Encounters were staged in two groups to

which the pairs of crabs were randomly allocated. In the control group, ( $N = 11$  pairs) the fight was terminated as the attacker made initial contact with the shell of the defender. This process ensured that control crabs were healthy individuals which were prepared to fight, but did not fight. The second group ( $N = 67$  pairs) contained fights which were allowed to proceed until their conclusion, where either the defender allowed itself to be evicted from its shell ( $N = 46$  pairs); or the attacker terminated the fight without first having evicted the defender ( $N = 21$  pairs).

Fights were staged between 0930 and 1200 hours each day. A large crab was placed in an arena consisting of a 95 mm diameter crystallizing dish divided in half by a piece of transparent acrylic, filled to a depth of 4 cm with aerated seawater, and containing a 1 cm thick sandstone tile as a base. The dish was then placed in an observational chamber, behind a two-way mirror, so that the crabs could not see the observer. The small crab was then introduced into the other half of the arena containing the large crab, and allowed to acclimate for 10 seconds before the transparent partitioning was removed. This ensured that both crabs were aware of each other before being allowed to physically interact. Once the partition was removed, the ensuing behaviours and pattern of shell rapping was recorded using a Psion Workabout MX (Psion Teklogix Inc., Mississauga, ON, Canada) configured as a time-event recorder using The Observer 5.0 (Noldus IT, Wageningen, The Netherlands) event-recording software. The parameters of the pattern of shell-rapping (contest duration in seconds, total number of raps, total number of bouts of rapping, mean number of raps/bout and mean pause duration in seconds) were then analysed in order to calculate the attacker vigour for each fight (see ethogram in Chapter 2 Materials and Methods).

Following shell fights, the crabs were fed, and kept individually for a 45 hour recovery period in order to accommodate the performance capacity trials (Chapter 4) before being used for muscle analysis.

### ***Abdominal muscle analysis***

In order to measure a proxy for the strength of hermit crabs the abdominal muscles, were analysed for protein content. After the performance capacity trials (see Chapter 4), each crab was humanely killed by immersion in liquid nitrogen and temporarily stored at  $-20^{\circ}\text{C}$ . The crabs were individually removed from the freezer and while still frozen the membranous abdomen was dissected away from the cephalothorax using a scalpel. The abdomen was

dissected, and the musculature was carefully removed from the other tissues present (epithelium, digestive tract and testes). The muscle tissue of each crab was weighed, then placed in a test tube with 2.0 ml of 0.5 M sodium hydroxide, and incubated in a water bath at 37°C for 2 hours. The samples were vortex mixed at the start, and then halfway through the incubation period. After a final thorough mixing, each sample was split into two Eppendorf tubes and centrifuged at 13000 r.p.m. for 3 min. The supernatant was carefully removed from each tube, and then combined in a labelled Eppendorf tube, which was vortex mixed and then stored at -20°C until all samples were collected.

Analysis of muscle protein concentration was based on the Bradford Method (Bradford 1976), where Bradford Reagent is added to samples and protein standard solutions (made at 0, 50, 100, 200, 400, 600 µg/ml) arranged in a 96 well microplate. Samples were removed from the freezer, and thawed on ice, after which 50 µl of each sample was pipetted into an Eppendorf tube, to which 1.0 ml of ultra pure water (Elga Maxima, High Wycombe, UK) was added. Three 10 µl portions of each sample and protein standard solution were placed in separate wells in seven 96-well microplates (i.e. 3 wells per sample). Then 250 µl of Bradford Reagent was added to each well. The samples were left to develop for 10 minutes, then placed into a plate reader (OPTImax tunable microplate reader, Molecular Devices, Sunnyvale, CA, USA) and read at 595 nm. The absorbances for the standard solutions were used to generate a regression from which the concentration of protein in each sample well could be calculated. The amount of muscle protein in mg/g was then calculated for each contestant by dividing the concentration of protein in each sample well by the muscle mass of each crab. In addition to the muscle protein concentration, the muscle/body weight ratio was calculated for each individual by dividing the abdominal muscle weight by the overall crab weight.

RESULTS

EFFECT OF FIGHTING

The concentration of muscular protein was not predicted to vary as a result of fighting or performance testing; however this was tested by performing a repeated measures ANOVA. The between subject factor in the model was fight status (fought or the unfought control group), while the repeated measure was the concentration of muscle protein (mg/g) in each role (attacker or defender).

The concentration of protein in the abdominal muscles of the crabs was not affected by fighting ( $F_{1,76} = 0.355, P = 0.5531$ ), and there was no difference in the concentration of muscle protein between the roles adopted by the crabs ( $F_{1,76} = 0.095, P = 0.7584$ ). A non-significant trend for an interaction effect was detected between the role adopted by the crabs and whether or not they fought. Fought attackers appear to have higher muscle protein than unfought control attackers, whereas fought defenders appear to have less muscle protein than unfought control defenders ( $F_{1,76} = 3.317, P = 0.0725$ ; Figure 5.1).

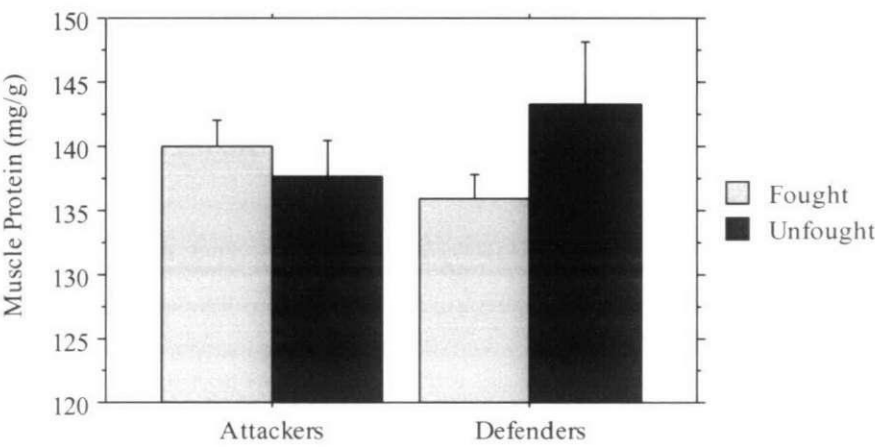


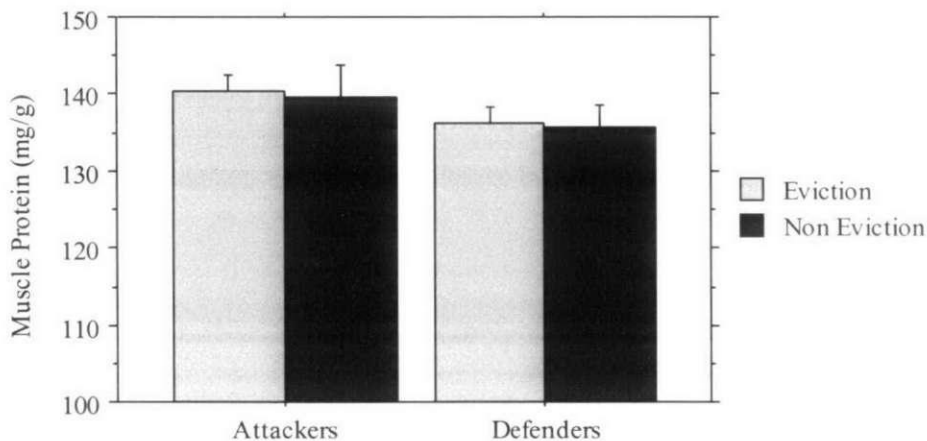
Figure 5.1. Muscle protein concentration in each role in relation to whether they fought.

## FIGHT OUTCOMES AND MUSCLE PROTEIN

### *Repeated Measures ANOVA – Differences in muscle protein between roles and outcomes*

As participating in a fight did not appear to affect the muscle protein concentration of the hermit crabs, the control group was excluded from further analysis. The following results thus focus on the differences between fight outcomes and roles. The effects of fight outcome on abdominal muscle quality were determined by performing repeated measures ANOVAs. The between subject factors in the models were outcome (eviction or non-eviction), while the repeated measure was either the muscle protein concentration or the muscle/body weight ratio in each role (attacker or defender).

There was no effect of fight outcome on muscle protein concentration ( $F_{1,65} = 0.024$ ,  $P = 0.8768$ ), although there was a non-significant trend for higher muscle protein in attackers than in defenders ( $F_{1,65} = 3.356$ ,  $P = 0.0715$ ). There was no significant interaction between the muscle protein concentration in each role and the outcome of the fight ( $F_{1,65} = 0.007$ ,  $P = 0.9353$  Figure 5.2).

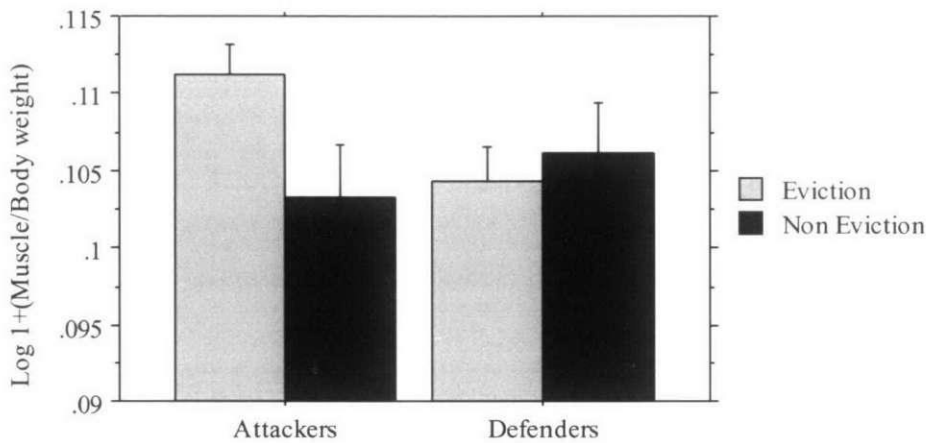


**Figure 5.2.** Muscle protein concentration in each role in relation to the outcome of the fight.

### *Repeated Measures ANOVA – Differences in muscle/body weight ratio between roles and outcomes*

No significant difference was detected in the muscle/body weight ratio between the fight outcomes ( $F_{1,65} = 0.973$ ,  $P = 0.3276$ ), or between the roles adopted by the crabs ( $F_{1,65} =$

0.895,  $P = 0.3476$ ). A significant interaction was found between the role adopted by the crab and the outcome of the fight on the muscle/body weight ratio ( $F_{1,65} = 5.155$ ,  $P = 0.0265$ ; Figure 5.3), where winners (attackers that effected evictions and defenders that resisted eviction) have higher muscle/body weight ratios than losers (attackers that failed to evict defenders and defenders that were evicted).



**Figure 5.3.** Muscle/body weight ratio for each role in relation to the outcome of the fight.

**ANCOVAs – Muscle protein concentration**

One-factor ANCOVAs were used to examine each role independently. The dependent variable in these models was muscle protein concentration, with the weight of the crabs as the covariate. The factor included in each model was fight outcome (eviction or non-eviction).

There were no significant interaction effects involving the covariate, and these were thus deleted from the models. When attackers were analysed on their own, no significant difference was detected in the muscle protein concentration between attackers that effect evictions and attackers that do not effect evictions ( $F_{1,64} = 0.261$ ,  $P = 0.6109$ ), and there was no relationship between the muscle protein concentration and the size of attackers ( $F_{1,64} = 0.988$ ,  $P = 0.3240$ ).

Similarly, when defenders were analysed on their own, no significant difference was found between the muscle protein concentration of evicted and non-evicted defenders ( $F_{1,64} = 0.014$ ,  $P = 0.9067$ ), and there was no relationship between the muscle protein concentration and the size of defenders ( $F_{1,64} = 0.013$ ,  $P = 0.9111$ ).

### *ANCOVAs – Muscle/body weight ratios*

Again, in order to investigate potential interaction effects, the muscle/body weight ratios for each role were analysed independently. The dependent variable in these models was the muscle/body weight ratio of the crabs and the factor included in each model was fight outcome (eviction or non-eviction). Crab weight, although included in the calculation of the muscle/body weight ratios, was used as a covariate as it may be related to the muscle/body weight ratio and to the outcome of the fights.

When attackers were analysed on their own, there was no significant effect involving the covariate, which was deleted and the model recalculated. In the recalculated model, no significant difference was detected in the muscle/body weight ratio between fight outcomes ( $F_{1,64} = 1.455$ ,  $P = 0.2321$ ), although there was a significant effect weight ( $F_{1,64} = 5.589$ ,  $P = 0.0211$ ).

When defenders were analysed on their own, a significant difference was found in the muscle/body weight ratio between the fight outcomes, with defenders that resisted eviction having higher muscle/body weight ratios than those that were evicted ( $F_{1,63} = 4.128$ ,  $P = 0.0464$ ). There was also a significant effect of defender weight on the muscle/body weight ratio ( $F_{1,63} = 35.283$ ,  $P < 0.0001$ ) and a significant two-way interaction between defender weight and outcome ( $F_{1,63} = 5.158$ ,  $P = 0.0266$ ).

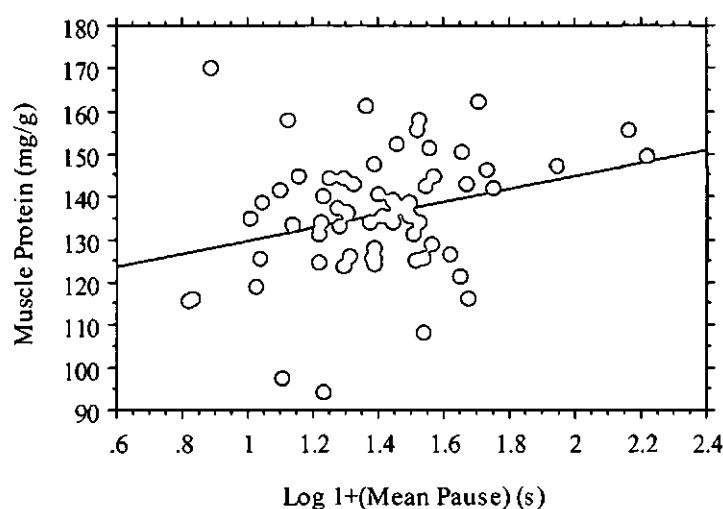
### **CORRELATIONS BETWEEN FIGHT VIGOUR AND MUSCLE QUALITY**

In order to determine how abdominal muscle composition may influence fight vigour, a series of one-factor ANCOVAs was performed that included either the muscle protein concentration (mg/g), or muscle/body weight ratio as the dependent variable, and fight variables associated with vigour as covariates. Fight outcome was included as a factor in the model as it has been shown to be influenced by the abdominal musculature of the contestants. Bivariate scattergrams with regression lines have been presented in order to illustrate the direction of the effects.

No significant effects were found between the muscle protein concentration and the pattern of shell rapping. In attackers there were no significant effects between muscle protein concentration and contest duration ( $F_{1,62} = 0.806$ ,  $P = 0.3723$ ), total raps ( $F_{1,62} = 0.191$ ,  $P =$

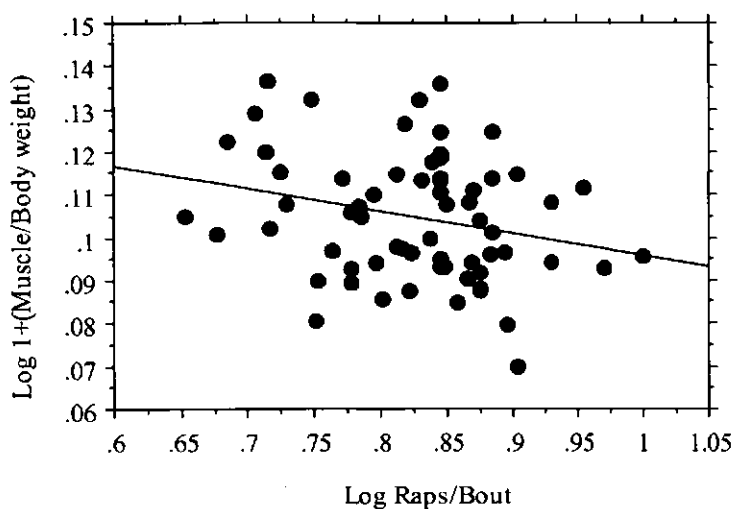


0.6639), total bouts ( $F_{1,62} = 0.149$ ,  $P = 0.7006$ ), raps/bout ( $F_{1,62} = 0.111$ ,  $P = 0.7402$ ), or mean pause duration ( $F_{1,62} = 1.034$ ,  $P = 0.3135$ ). Similarly, in defenders there were no significant effects between muscle protein concentration and contest duration ( $F_{1,62} = 1.583$ ,  $P = 0.2130$ ), total raps ( $F_{1,62} = 0.650$ ,  $P = 0.4231$ ), total bouts ( $F_{1,62} = 0.843$ ,  $P = 0.3622$ ), and raps/bout ( $F_{1,62} = 0.695$ ,  $P = 0.4076$ ). However, a significant positive relationship was detected between the abdominal muscle protein concentration of defenders, and the mean pause duration performed by the attacking crabs ( $F_{1,59} = 5.250$ ,  $P = 0.0255$ ; Figure 5.4).



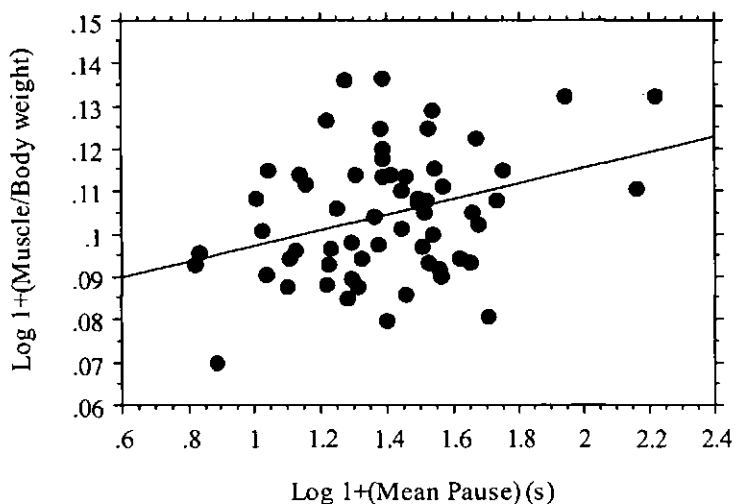
**Figure 5.4.** Muscle protein concentration in defenders in relation to the mean pause duration.

As for muscle protein, there were no significant effects between the muscle/body weight ratios of attackers and the contest duration ( $F_{1,62} = 0.535$ ,  $P = 0.4673$ ), total raps ( $F_{1,62} = 0.981$ ,  $P = 0.3259$ ), total bouts ( $F_{1,62} = 0.859$ ,  $P = 0.3576$ ), raps/bout ( $F_{1,62} = 0.015$ ,  $P = 0.9028$ ), or mean pause duration ( $F_{1,59} = 0.004$ ,  $P = 0.9517$ ). In defenders, no significant effects were detected between the muscle/body weight ratio and contest duration ( $F_{1,62} = 2.603$ ,  $P = 0.1118$ ), total raps ( $F_{1,62} = 1.216$ ,  $P = 0.2743$ ), or total bouts ( $F_{1,62} = 2.127$ ,  $P = 0.1498$ ). However, a significant negative relationship was detected between the muscle/body weight ratio of defenders and the number of raps/bout ( $F_{1,62} = 4.028$ ,  $P = 0.0491$ ; Figure 5.5).



**Figure 5.5.** Muscle/ body weight ratios of defenders in relation to the number of raps/bout.

A significant positive relationship was also found between the muscle/body weight ratios of defenders and the mean pause duration performed by the attacking crabs ( $F_{59,1} = 7.620$ ,  $P = 0.0077$ ; Figure 5.6).



**Figure 5.6.** Muscle/body weight ratios of defenders in relation to the mean pause duration.

## DISCUSSION

Muscle quality in terms of protein concentration did not vary between the fight outcomes (Figure 5.2) whereas the muscle/body weight ratio was found to be higher in winners than losers, (i.e. attackers which evicted defenders, and defenders which resisted eviction; Figure 5.3). However, larger muscle/body size ratios appear to be more important to the competitive success of attackers than defenders. Although the interaction illustrates that winners appear to have larger muscle/body weight ratios than losers (Figure 5.3), there is no significant difference between the muscle/body weight ratios of attackers that won (those that evicted defenders) and attackers that lost (those that failed to evict defenders). However, successful defenders that retained their shells had significantly larger muscle/body ratios than unsuccessful defenders that were evicted. Furthermore, overall crab weight was found to affect the outcome of shell fights, being higher in winners (attackers that effected evictions and defenders that resisted eviction). This suggests that relative muscle size is more important to defending crabs, whereas overall size may be more important to attacking crabs in shell fights. A significant positive correlation was detected between the abdominal muscle protein concentration of defenders, and the length of pauses between bouts of shell rapping performed by the attackers (Figure 5.5). It is highly unlikely that muscle protein changes in response to the pattern of shell rapping received by the defender, and indeed, muscle protein did not change in either role as a result of fighting and performance testing (Figure 5.1). Instead, attackers may adjust the pattern of shell rapping in response to defender quality. A similar positive relationship was found between the length of pauses performed by attackers and the muscle/body weight ratios of defenders (Figure 5.6). In addition, the abdominal muscle/body weight ratios of defenders were found to be negatively correlated with the number of raps/bout performed by attackers (Figure 5.5). Again, this may reflect the response of the attacker to defender quality. Either attackers that perceive weaker defenders rap more vigorously in an attempt to evict them quickly, or good quality defenders exhaust attackers, leading to less vigorous rapping (i.e. longer pauses and fewer raps/bout).

In the absence of a direct measurement for strength in hermit crabs, muscle quality was used as a proxy, similar to many physiological variables that have been used as proxies for stamina (e.g. *L*-lactate, glucose, glycogen and respiratory pigments). The concentration of muscular protein was not found to affect the outcome of shell fights (Figure 5.2) whereas larger muscle/body weight ratios increased the chances of winning, but only in defenders (Figure 5.3).

It would be predicted that attackers would benefit most from larger abdominal muscles as they perform the more obviously demanding behaviour by engaging in shell rapping, in which they must lift their own shell and forcefully hit it repeatedly against that of the defender. Further, they must attempt to pull the defender from its own shell during breaks in the shell rapping pattern. It appears counterintuitive therefore that there was no difference in the muscle/body weight ratio between the outcomes for attackers. However, it appears that overall size is more important in contributing to an attacker's chances of success in shell fights.

In contrast, the muscle/body weight ratio was found to vary between successful and unsuccessful defenders (Figure 5.3). As defenders must hold on to the contested shell, they would also be predicted to gain an advantage from better quality abdominal muscles. Whilst attackers use their abdominal muscles more frequently throughout fights than defenders as they perform shell rapping, this activity is sustained, and may rely on stamina more than on strength. Larger abdominal muscles may thus be more beneficial to defending crabs, which must grip tightly onto their shell during the points at which the attacker attempts to evict them. Defenders may therefore rely more on bursts of strength (i.e. defensive muscle contraction), which may correlate with muscle size. This may be a similar mechanism to that demonstrated in Chapter 4, wherein speed predicted fight outcome in defenders but not attackers, whereas stamina predicted fight outcome in both defenders and attackers. A potential solution would be to carry out a similar experiment, but to quantify the relative proportion of fast and slow (phasic and tonic) muscle fibres (e.g. Fahrenbach 1967; Ogonowski & Lang 1979) or the innervation of phasic and tonic motoneurons (e.g. Atwood 1973; Chapple 1993) between outcomes and in each role.

In contrast to the findings on the muscle/body weight ratios, muscle quality in terms of protein concentration does not vary between the winners and losers of shell fights. Thus, muscle size is more important in influencing RHP in hermit crab shell fights than muscle quality.

In addition to the effects of defender muscle size on the outcome of shell fights, the pattern of shell rapping was related to both the defenders muscle protein concentration (Figure 5.4) and muscle/body weight ratios (Figures 5.5 and 5.6). It appears that better quality muscles are negatively correlated with the vigour of shell rapping, and there are two possible explanations for these relationships. First, better quality defenders may be more difficult for the attacking crabs to evict. Here, fights would continue for longer, and the attackers would begin to suffer the effects of fatigue, which would reduce the vigour of rapping, leading to

fewer raps/ bout (Figure 5.5) and longer pauses (Figures 5.4 and 5.6). However, there was no significant relationship between defender muscle protein and contest duration. An alternative explanation is that the attackers adjust the vigour of shell rapping based on the perceived quality of the defender. Here, if a weak defender is perceived, attackers may rap more vigorously in an attempt to evict the defender more quickly from the shell. However, if an attacker perceived an overly strong defender, it would be better to terminate the fight rather than to persist in a difficult encounter. This finding provides evidence that attackers are able to assess the quality of defenders, a process which was previously assumed to be unlikely as defenders remain tightly withdrawn inside their shells for the duration of the encounter. Thus, it appears that attackers are able to gain information about the defender and then modify the pattern of shell-rapping accordingly.

The present data demonstrate that not all of the factors assumed to be important to contest behaviour may actually be so. Muscle protein concentration does not affect fight outcomes, but an associated variable (muscle/body weight ratio) does. This suggests that analysing multiple associated variables may provide greater insight into the mechanisms governing agonistic success than a single factor may (e.g. glucose and glycogen, Briffa & Elwood 2001; Briffa & Elwood 2004). Furthermore, the analysis of single physiological proxies may not effectively reveal why individuals perform at certain levels during contests, whereas whole organism performance capacity experiments may provide a greater insight. However, using a combination of surrogate measures, this study was able to infer that strength was important to defending hermit crabs, but not to attacking ones. As attackers engage in the repeated signalling process of shell rapping, increased stamina may be more important, whereas defenders require the strength to resist the attackers' attempts at eviction.

## **CHAPTER 6**

**Personality or performance? Behavioural syndromes,  
aerobic capacity and fighting ability in the hermit crab  
*Pagurus bernhardus*.**

## ***Abstract***

Differences in aggressive behaviour can be understood by analysing animal fights from a proximate, causal perspective. This can range from the analysis of energetic metabolites associated with performance as well as chemical control mechanisms, to whole-organism performance capacities. However, another whole-organism approach is to analyse individual variation in terms of consistent individual differences in behaviour. Where these differences correlate, either temporally or across behavioural contexts, they are termed 'behavioural syndromes' or 'animal personalities'. This study examined the behaviour of hermit crabs across three contexts (boldness, exploration and aggression) and two situations (low predation risk and high predation risk), as well as in relation to the outcome of shell fights. In order to obtain a measurement of the 'state' of each individual crab, their haemolymph haemocyanin concentration ( $[Hc]_{HL}$ ) was also measured as this has been shown to determine the activity levels and fighting ability in crustaceans. 'Behavioural syndromes' consistent with a shy – bold continuum were identified for the hermit crab *Pagurus bernhardus*, although these were not found to predict fighting ability. The  $[Hc]_{HL}$  did not influence the behavioural syndromes, but did affect the ability to win fights, with successful attackers that evicted defenders having higher  $[Hc]_{HL}$  and higher Hc residuals than unsuccessful attackers that failed to effect evictions. This study thus demonstrates that although behavioural syndromes exist in hermit crabs, it is their physical capacity rather than their 'personality' that affects the ability to win fights.

## INTRODUCTION

Animal contests are often analysed with respect to variation in factors such as morphology, e.g. body size (Caldwell & Dingle 1979; Riechert 1978), weapon size (Barki et al. 1997; Sneddon et al. 1997), ornamentation (Lailvaux et al. 2005; Vanhooydonck et al. 2005), physiology, (e.g. energy reserves, see Briffa & Elwood 2004; or metabolic by-products, see Neat et al. 1998); aerobic capacity (see Chapter 3) or whole body correlates such as performance capacities (e.g. Brandt 2003; Perry et al. 2004), which may be related to resource holding potential (RHP). However, in addition to constraints on performance, agonistic behaviour may be influenced by cognitive or 'psychological' factors such as 'animal personalities' (sensu Dall et al. 2004; Sih et al. 2004).

Individual variation is recognised in many phenotypic traits exhibited in animals (see Dall et al. 2004 and references therein), yet variation in behaviour has until recently been assumed to represent non-adaptive 'noise' surrounding an adaptive mean (Coleman & Wilson 1998; Dall et al. 2004). Such individual variation, however, may be non-random in its distribution along behavioural axes (Gosling 2001), and is likely to have ecological consequences (Dall et al. 2004). The proposed mechanism driving such differences in behavioural phenotypes is a limitation to behavioural flexibility. This is contrary to the concept of behaviour being the most flexible aspect of phenotype (Hazlett 1995), and suggests that behavioural flexibility may either be limited, or potentially costly to the individual. Where flexibility is limited, this may occur through proximate constraints such as limits on sensory capabilities, learning abilities, cognitive structure and morphology (Hazlett 1995), all of which influence the processes of receiving and appropriately responding to stimuli. Intrinsic costs associated with the maintenance of the structures in this system may also impose limits on flexibility if such costs are sufficiently high (Briffa et al. 2008b). Alternatively, costs may be incurred where plasticity causes animals to make 'unavoidable errors', leading to inappropriate responses to stimuli (Dall et al. 2004; Sih et al. 2004). Thus, behaving consistently may be preferable, generating 'approximately appropriate' (Briffa et al. 2008b) responses, and leading to consistent individual differences in behaviour. In animals, these consistent differences may be correlated temporally or across situations, where they are termed 'animal personalities' (Gosling 2001) and different behaviours may be correlated between functional contexts to produce 'behavioural syndromes' (Sih et al. 2004). In addition, these syndromes may be stable temporally and across situations.



Behavioural syndrome research has shown that the strength of particular behaviours exhibited by an individual (or its 'behavioural type', Bell 2007) can form a continuum along various axes, the best known of which is the shy – bold continuum (Wilson et al. 1993) or simply 'boldness'. Here, individuals differ in their willingness to take risks (Brick & Jakobsson 2002; Sinn et al. 2008), with 'bold' individuals exhibiting a high degree of exploratory behaviour, interest in novel objects (Réale et al. 2000) and short 'startle responses' when disturbed (Briffa et al. 2008b), whereas 'shy' individuals exhibit longer startle responses. A similar axis is the proactive – reactive axis (Koolhaas et al. 1999). In this instance, proactive individuals are bold and aggressive, and actively perform exploratory behaviour as well as forming routines, whereas reactive individuals respond cautiously to stimuli (Sih et al. 2004), leading to the development of different 'coping styles'.

A number of studies have demonstrated consistent differences in boldness between populations of a species (Briffa et al. 2008b; Wilson et al. 1993) as well as between individuals within populations (Briffa et al. 2008b; Wilson 1998). For example, behavioural syndromes have been described in mammals such as bighorn sheep (*Ovis canadensis*) where ewes consistently differ in their docility (Réale et al. 2000), and in birds such as western bluebirds (*Sialia mexicana*), where a syndrome of aggression was found across the contexts of male-male aggression and nest defence (Duckworth 2006). Similarly, in the streamside salamander (*Ambystoma barbouri*) behaviours in the contexts of foraging and antipredator responses are correlated (Sih et al. 2003). Behavioural syndromes have also been found in various species of fish, for example, in the pumpkinseed sunfish (*Lepomis gibbosus*), boldness is related to diet, parasite loading and risk of predation (Wilson 1998; Wilson et al. 1993) and in the rainbow trout (*Oncorhynchus mykiss*) where boldness is related to learning ability (Sneddon 2003).

Behavioural syndromes are not confined to the vertebrates, however, with syndromes being identified in spiders and fiddler crabs. Chadwick Johnson and Sih (2005) found a syndrome spanning the contexts of aggression, feeding and courtship in the cannibalistic fishing spider (*Dolomedes triton*), and a similar syndrome was found by Reaney and Backwell (2007) in the fiddler crab *Uca mjoebergi*, where boldness, aggression, exploratory behaviour and courtship were found to be correlated. Furthermore, consistent individual differences in boldness have been found between and within populations of the hermit crab *Pagurus bernhardus* (Briffa et al. 2008b), and over the developmental stages of squid (Sinn et al. 2008).

Behavioural syndromes may help to explain the mechanisms behind systems such as dominance hierarchies (Dall et al. 2004) and alternative mating strategies, whereby individual variation is the result of natural selection rather than the raw material that it operates on (Sloan Wilson et al. 1993). Similarly, behavioural syndromes may explain individual differences in aggression (Huntingford 1976; Riechert & Hedrick 1993) and the existence of distinct personality types may make particular game theoretical models more realistic. For example, in the Hawk-Dove game (Maynard Smith & Price 1973), two distinct behavioural ‘types’ are utilised in order to demonstrate how fighting by convention is predicted when the costs of engaging outweigh the value of the resource. The ‘Hawk’ phenotype always escalates whereas the ‘Dove’ only displays, and retreats if the opponent escalates. The roles can either be maintained by competitors switching between strategies, or by individuals maintaining consistent strategies. Until the relatively recent research into behavioural syndromes, it was thought that the likely solution was that individuals switched strategies in a frequency-dependent manner. However, as consistent individual differences are now known to exist in nature, an interpretation of the Hawk-Dove game based on consistent behavioural types is possible (Dall et al. 2004).

Several empirical studies have demonstrated that individuals can be consistent in their levels of aggression, and also that aggression is correlated with boldness in species such as three-spined sticklebacks, *Gasterosteus aculeatus* (Huntingford 1976), South American cichlids, *Nannacara anomala*, (Brick & Jakobsson 2002) and funnel web spiders, *Agelenopsis aperta*, (Riechert & Hedrick 1993). This is consistent with ‘domain general’ (rather than ‘domain specific’ or ‘context specific’) behavioural syndromes (see Réale et al. 2000), but an alternative view is that aggression and fear are governed by separate physiological and behavioural mechanisms (Riechert & Maynard Smith 1989). Brick and Jakobsson (2002) analysed fights between the South American cichlid *N. anomala* in two different situations which varied in their level of predation risk. It is necessary to observe behaviour either at different times or between different situations in order to see if the responses of individuals change, and if so, whether the magnitude of these responses remain consistent relative to those of other individuals in the study population (Briffa et al. 2008b; Sih et al. 2004). Bold dyads were shown to escalate more quickly to dangerous fighting behaviours (such as mouth wrestling) than shy dyads, as well performing these behaviours more often. Furthermore, bold dyads fought more readily in the presence of a model predator (high predation risk situation) than did shy dyads. This study demonstrates that fighting

asymmetries may also include an individual's perception of risk (Brick & Jakobsson 2002) or 'boldness' in animal personality terms.

Another aspect of contest behaviour where individual differences may be important is when animals aggressively defend territories. Here, consistent and predictable behaviour would allow individuals to recognise the risk associated with challenging particular opponents (see Dall et al. 2004; Sih et al. 2004). A study by Hyman & Hughes (2006) demonstrated that territorial male song sparrows (*Melospiza melodia*) differentiate between aggressive and non-aggressive neighbours, and respond more strongly to aggressive neighbours. Unlike the Hawk-Dove game with eavesdropping (Dall et al. 2004), territory holders appear to be behaving adaptively by responding more aggressively to individuals that pose a greater threat to their territory (Hyman & Hughes 2006). In this case, an intruder is attempting to take the territory from the owner. This is similar to other 'asymmetrical' contest systems where one opponent attempts to take a resource from its owner, such as the guarded mates of rival males in species such as amphipods (Dick & Elwood 1996), damselflies (Cordero 1999) and newts (Verrell 1986); burrows and their resident females from rival males in dung beetles (see Lailvaux et al. 2005 and references therein); shelters from their occupiers in stomatopods (Shuster & Caldwell 1982); and gastropod shells from their occupiers in hermit crabs (Elwood & Briffa 2001).

Hermit crab shell fights occur over the ownership of the gastropod shells that protect the uncalcified membranous abdomen of the crabs. During the fights, the opponents adopt two very different roles: 'attacker' and 'defender'. The attacking crab initiates the encounter and attempts to take the gastropod shell of the non-initiator, which attempts to defend its shell by withdrawing tightly into it. The attacker overpowers the defender and then performs a repetitive signalling behaviour termed 'shell-rapping'. The attacker grips the shell of the defender closely with its walking legs, and begins to bring its own shell repeatedly into contact with that of the defender as a bout of raps in quick succession. A series of such bouts, separated by distinct pauses are performed during the shell fight. Shell fights can end in one of two ways, either the defender decides to give up; allowing itself to be evicted by the attacker, which removes the defender through the aperture of the shell; or the attacker may decide to give up without having first evicted the defender. The vigour of shell-rapping is a key determinant of the outcome of shell fights, with attackers that perform more frequent (Briffa et al. 1998) and more powerful raps (Briffa & Elwood 2002) being more likely to evict defenders. This suggests that the pattern of rapping contains information about fighting ability, advertising the attacker's stamina (Briffa & Elwood 2000a; Briffa & Elwood 2000c;



The aims of this study were to identify whether behavioural syndromes occurred in the hermit crab *P. bernhardus*, whether they were stable between situations (Figure 6.1) and whether they affected the outcome of contests, using shell fights as a model system. To determine the stability of a syndrome, the principle components (PC) scores for potentially correlated behaviours were compared between two situations. Although there may still be behavioural flexibility between situations, the relative importance of personalities can be determined by comparing effect size estimates for tests of correlation and difference in PC scores between situations (see Briffa et al. 2008). Three behavioural contexts were chosen for investigation: (i) boldness, (ii) exploratory behaviour, and (iii) aggression. As several studies suggest that an animal's 'state' (including its internal state, i.e. physiology) may influence the formation of behavioural syndromes (e.g. Koolhaas et al. 1999; Rands et al. 2003; Riechert & Hedrick 1993), a further aim of this study was to examine how a measure of overall physiology known to influence fighting ability (haemolymph haemocyanin concentration ([Hc]<sub>HL</sub>), Chapter 3) may influence RHP and any behavioural syndromes identified.

## MATERIALS AND METHODS

### *Study organisms*

Specimens of the common European hermit crab *Pagurus bernhardus* were collected between April 2008 and July 2008 from rock pools at Hannafore Point, UK (50° 20' N, 4° 27' W). The collected crabs were restricted to a size range sufficient for the extraction of adequate haemolymph samples for analysis (crab weight = 0.570 – 1.650 g).

The hermit crabs were kept in groups of 80-180 in 65 cm x 50 cm x 30 cm plastic tanks, filled with aerated seawater to a depth of 15 cm (~50 l). These were maintained at 15°C in a temperature controlled laboratory. The crabs were fed on a diet of catfish pellets and were returned to the shore within a fortnight of collection.

### *Shell allocation*

Hermit crabs were removed from their gastropod shells by cracking the shells open in a bench vice. They were then sexed as only male crabs were used in order to avoid sex-related behavioural differences as found in previous studies (Neil & Elwood 1985). Study animals were examined for physical damage, and only crabs which were (i) free from obvious parasites, (ii) undamaged and (iii) intermoult were used in the experimental trials. Unused crabs were provided with new shells and were returned to the site from which they were originally collected.

The study crabs were assigned to pairs consisting of a small crab and a large crab (mean weights  $\pm$  s.e.; small,  $0.830 \pm 0.022$  g; large,  $1.048 \pm 0.027$  g). The larger crab of the pair (potential attacker) was provided with a *Littorina littorea* shell that was only 50% of its preferred shell weight, while the smaller crab (potential defender) was provided with a *L. littorea* shell which would be 100% adequate for the larger crab of the pair. The preferred shell size was determined using a regression that related crab weight to preferred shell weight (Briffa & Elwood 2007).

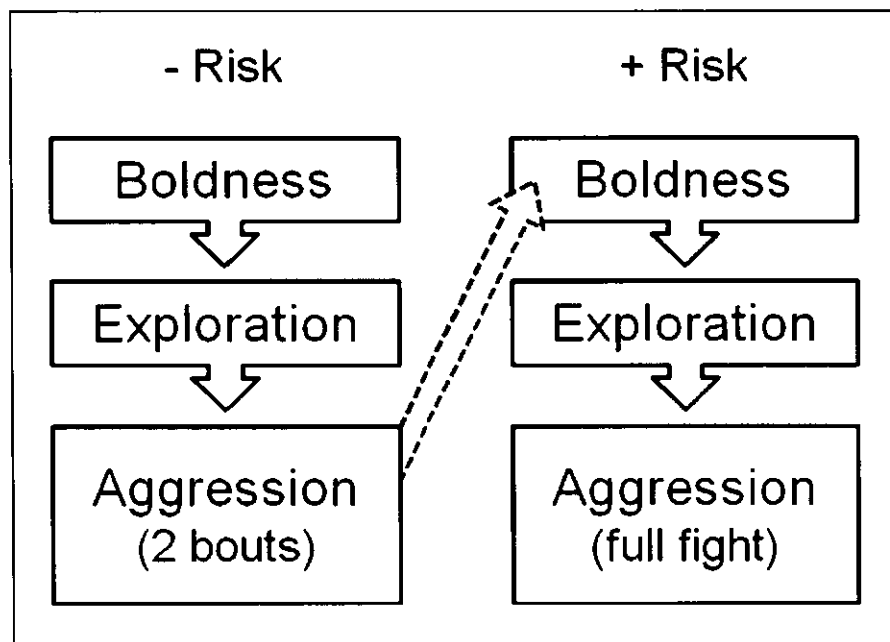
The disparity in size between the contestants was kept similar for each trial by calculating the relative weight differences (RWD) between the crabs before assigning them to pairs. These were calculated following Briffa et al. (1998):

$$\text{RWD} = 1 - (\text{weight of small crab} / \text{weight of large crab})$$

Thus, a greater RWD reflects a greater disparity in size between the contestants. The RWD of the pairs in the experiment ranged from 0.150 g to 0.259 g (mean RWD  $\pm$  s.e = 0.209  $\pm$  0.003g).

### *Analysis of behaviours between situations and contexts*

In order to test whether the behaviour of hermit crabs was correlated across situations and contexts, the behaviours of the hermit crabs were recorded in three trials that corresponded to the ‘boldness’, ‘exploratory’ and ‘aggressive’ contexts. The crabs were then rested and the trials repeated with the same hermit crabs, but in a different situation. The situations used were a high predation risk situation, and a low predation risk situation, which were created by using predation cue water or control water. Thus, each crab completed trials in three contexts across two situations, and in order to control for the timing of the situation received, half of the crabs were exposed to the low risk situation first, while half were exposed to the high risk situation first (see Figure 6.2).



**Figure 6.2.** Diagram illustrating the experimental design.

Following shell allocation, crabs were isolated in 95 mm diameter crystallizing dishes, and assigned to one of two treatment groups corresponding to two predation risk situations: low predation risk (- cue), or high predation risk (+ cue). The individuals exposed to the low predation risk situation were placed into crystallizing dishes filled with aerated seawater whereas the individuals exposed to the high predation risk situation were placed into crystallizing dishes filled with aerated predation cue water. This was taken from a tank containing 3 l of seawater in which two male *Carcinus maenas* of 50-80 mm carapace width had been maintained for 24 hours. Previous studies show that hermit crabs respond to the chemical presence of these crabs (Briffa et al. 2008a; Rotjan et al. 2004). The hermit crabs were allowed to acclimate to these conditions for 16 hours prior to being used in behavioural observations.

#### ***Startle responses (Boldness test)***

Startle response trials were carried out between 0900 and 0930 hours each day. Such measures have been used previously to assess the motivation of hermit crabs to fight (Elwood et al. 1998) and to investigate shells (Jackson & Elwood 1990) by dropping a piece of black card over the arena that the crabs were being observed in. In contrast, the startle responses in this experiment were used to gain information about the boldness of the individual and were carried out following Briffa et al. (2008). The crab was lifted out of its crystallizing dish and held in an inverted position by the observer for a period of 10 seconds before being placed aperture-upwards in a crystallizing dish containing either aerated seawater (- cue) or predator cue water (+ cue) corresponding to the treatment that the focal crab had received in its overnight holding dish. The crab was placed in one half of the dish, which was divided in half by a piece of opaque acrylic, and contained a 1 cm thick sandstone tile as a base. This process of inverting the crab caused the crabs to withdraw fully into their shells. The duration of this 'startle response' was timed from the point at which the crab was placed onto the substrate of the arena until it had re-emerged such that it had placed both pairs of walking legs onto the substrate. The duration of the startle response was recorded using a Psion Workabout MX (Psion Teklogix Inc., Mississauga, ON, Canada) configured as a time-event recorder using The Observer 5.0 (Noldus IT, Wageningen, The Netherlands) event-recording software.

#### ***Inquisitiveness (Exploratory behaviour test)***



After each boldness trial, the arena dish was placed in an observational chamber, behind a two-way mirror, so that the crabs could not see the observer. The piece of opaque acrylic was then removed to reveal a shell of 75% adequacy for the focal crab in the other side of the arena. Each new shell used in the trials had its aperture blocked with plasticine to prevent the crab from changing shells, similarly to the method used by Jackson & Elwood (1989) where dental cement was used to smoothly block the aperture of the shell. However, in this study the plasticine was inserted deep into the first whorl of the shell such that it would not be initially observed by the crab, leading to shell investigation. Inquisitiveness was measured by recording the following behaviours: Searching: (a) latency to move towards the new shell, (b) latency to contact the new shell; Manipulation: (c) amount of time spent moving and turning the new shell (external assessment), (d) amount of time spent with chelipeds inside the shell aperture (internal assessment); Entry: time of attempted entry of new shell. Similarly to the startle response, such behaviours have previously been used to assess the motivational state of the focal crab for obtaining a new shell (Jackson & Elwood 1989; Jackson & Elwood 1990). However, here these measures were used to gain information about traits intrinsic to the individual rather than perceived resource value, which was kept as similar as possible for each individual by following the regression that related crab weight to preferred shell weight presented in Briffa & Elwood (2007). All of these behaviours were recorded using the Psion Workabout MX.

### ***Shell fights (Aggression test)***

Following the inquisitiveness trials, the blocked 75% adequate shell was removed, and the arena was once again divided by the opaque acrylic. The small crab assigned to the pair was then introduced into the other half of the arena containing the large crab, and allowed to acclimate for 10 seconds before the partition was removed. This ensured that the potential defender had recovered from its own startle response and was active before the crabs were allowed to interact physically. Once the partition was removed, the ensuing pre-fight displays (see Figure 6.3) and contact sequence (latency to contact defender's shell, latency to insert chelipeds into contested shell's aperture, latency to rap) were recorded for the attacker using the Psion Workabout. The pattern of shell rapping for the first two bouts of rapping was also recorded using the Psion Workabout MX. The parameters of the pattern of shell rapping

### PRE-FIGHT DISPLAYS

Cheliped presentation	Crab extends chelipeds such that the manus of the claw is held at a right angle, with the claw tips resting on the substrate.
Cheliped extension	Crab rapidly extends chelipeds and holds them horizontally above the substrate.
Ambulatory raise	Crab raises a walking leg off the substrate and holds it up.
High posture	Crab raises itself off the substrate, assuming a high posture, often using the sides of the arena to do so.

(adapted from Arnott & Elwood 2007; Elwood & Neil 1992)

**Figure 6.3.** Ethogram of pre-fight display behaviours.

(number of raps/bout and pause duration in seconds) were then analysed in order to calculate the attacker vigour for the fight (see ethogram in Chapter 2 Materials and Methods). Only the first two bouts of shell rapping were recorded here before terminating the fight as the energetic expenditure incurred could affect the crabs' behaviour in the second situation.

#### *Recuperation and exposure to the alternative situation*

Following the artificially terminated shell fights, the crabs were fed, and returned to their individual holding dishes for a 24 hour recovery period before being exposed to the second situation such that half of the study animals were observed under the control conditions first followed by the predator cue, while the other half were observed under the predator cue treatment first followed by the control.

The startle responses, inquisitiveness, and shell fights were repeated under the second situation, although this time shell fights were allowed to proceed until their conclusion, where either the defender allowed itself to be evicted from its shell ( $N = 45$  pairs); or the attacker terminated the fight without first having evicted the defender ( $N = 20$  pairs). The outcome data could not be obtained for the first situation too as prior experience and the physiological

costs of engaging could have affected the behaviour of the focal crabs in subsequent trials. However, as each batch of crabs examined contained equal numbers of low predation risk (- cue) and high predation risk (+ cue) individuals, the outcome data were balanced.

### *Analysis of [Hc]<sub>HL</sub>*

After completing both situations, the crabs were again removed from their shells by cracking them open in a bench vice. A 20  $\mu$ L haemolymph sample was then taken from each crab by piercing the arthroidal membrane at the base of the third pereopod with a 29 gauge BD Micro-Fine™ 1 mL insulin syringe. The [Hc]<sub>HL</sub> was estimated using a well-established spectrophotometric method (see Chapter 3, Nickerson & Van Holde 1971; Spicer & Baden 2000).

### *Statistical methods*

Behavioural data relating to each of the 3 contexts under investigation (boldness, exploration and aggression) were collected for each situation (low predation risk and high predation risk) and log transformed to meet the requirements of the parametric tests. Each pair of variables was then tested for association between situations using correlation Z-tests, and for difference between situations using paired *t*-tests. As multiple comparisons were made using the same dataset, each was subjected to sequential Bonferonni corrections to P-values. The new (required) *P*<sub>2</sub> is included in the tables where this is the case, as is the new level of significance (S or NS). All of the results for this exploratory analysis are presented together with their effect size estimates in the Appendix in tables A.1 and A.2 (*r* for the correlations and Cohen's *d* and *r* values for the *t*-tests) in order to determine the relative importance of behavioural flexibility and consistency. These were calculated thus:

Cohen's *d*:

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sigma_{\text{pooled}}}$$

$$\text{where } \sigma_{\text{pooled}} = \sqrt{[(\sigma_1^2 + \sigma_2^2) / 2]}$$

$$r_{Y\lambda} = d / \sqrt{(d^2 + 4)}$$

The original (unlogged) behavioural variables were then analysed by principal components analysis (PCA) in order to identify related suites of behaviours that could represent behavioural syndromes. It was assumed that significant principal components (with Eigen values > 1) indicated the presence of a syndrome of correlated behaviours. Once such principal components had been identified these were tested for stability across the predation risk situations by correlation *Z*-tests and *t*-tests, and are included in Table 6.2 along with their effect size estimates (*R* for the correlations and Cohen's *d* and *r* values for the *t*-tests). However, such analyses were only performed for principal components that were qualitatively similar between situations. These factors were identified by comparing the magnitude and direction of each component (positive or negative) to ensure that they were similar in each of the situations.

The relationships between physiology and behaviour were then analysed by using correlation *Z*-tests to test for correlations between behavioural syndromes and [Hc]<sub>HL</sub>, and again the required *P*-values were Bonferroni corrected for these multiple comparisons. Both principal components factors and the [Hc]<sub>HL</sub> were then tested for difference between the fight outcomes using ANOVAs and ANCOVAs (see Results section for explanations of the models used).

All statistical tests were conducted using StatView 5.0 (SAS Institute Inc, Cary, NC, USA). All data were log transformed in order to normalise, however the PCA was conducted using the raw (unlogged) data.

## RESULTS

### *Correlations and paired t-tests for individual behaviours*

Following Bonferroni corrections to the required  $P$ -values, there were no significant correlations or differences between the situations (low predation risk and high predation risk) for any of the behaviours except for the startle responses (see Tables A.1 and A.2), which were positively correlated between the situations ( $Z = 7.752$ ,  $P < 0.0001$ ).

### *Principal Components Analysis*

The original (unlogged) behavioural data were analysed for behavioural syndromes by factor analysis. The number of variables included in the univariate tests (21, see Table A.1) had to be reduced prior to the PCA as not all individuals performed all the behaviours (especially displays), leading to missing values in the dataset. Thus, 10 variables were included in the PCA (Table 6.1). These were: startle response duration, latency to move in exploratory trials, latency to contact shell in exploratory trials, total time investigating the shell (contact + rotation + insertion), total display duration (presentations + extensions + ambulatory raises + high postures), latency to contact the defender's shell during fights, latency to insert chelipeds into the defender's shell, latency to first rap, the mean number of raps/bout, and pause duration.

The PCA detected relationships among the behavioural variables, generating four principal component factors with Eigen values  $> 1$ . However Factors 1 and 2 (PC1 and PC2) appear qualitatively to be the most stable between situations in the PCA, with most behaviours represented at similar magnitudes across the situations (see Table 6.1). PC1 positively relates the initial fighting behaviours (latency to contact, cheliped insertion and rapping) with the total duration of displays and the latency to contact the shell in the exploration context. PC2 positively relates startle responses with exploratory behaviours (latency to movement and latency to contacting the shell). However, in the low risk situation, these behaviours are negatively related with the duration of agonistic displays, whereas in the high risk situation they are positively related. In addition, in the high risk situation, the number of raps/bout is negatively correlated with the startle responses, exploratory behaviours (latency to movement and latency to contacting the shell) and display duration whilst this trend is absent in the low risk situation.

## Low Risk

### Factor Analysis Summary

Number of Variables	10
Est. Number of Factors	5
Number of Factors	5
Number of Cases	54
Number Missing	13
Degrees of Freedom	54
Bartlett's Chi Square	766.002
P-Value	<.0001

### Eigenvalues

	Magnitude	Variance Prop.
Value 1	3.498	.350
Value 2	1.340	.134
Value 3	1.202	.120
Value 4	1.074	.107
Value 5	.960	.096

### PCA Axes

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
- Startle	.226	.588	.176	.110	-.267
- Movement	.176	.548	-.444	-.488	-.174
- Contact (Lat)	.469	.595	.249	-.131	.247
- Total INV	-.205	-.163	-.308	-.458	.663
- All displays	.444	-.513	-.035	-.258	-.453
- Contact (Fight)	.970	-.084	-.091	.138	.126
- Insert (Fight)	.971	-.090	-.095	.134	.124
- 1st Rap	.965	-.088	-.114	.141	.138
- Raps/Bout	.072	-.073	.840	-.148	.234
- Mean Pause	-.367	.148	-.281	.672	.216

## High Risk

### Factor Analysis Summary

Number of Variables	10
Est. Number of Factors	5
Number of Factors	5
Number of Cases	60
Number Missing	7
Degrees of Freedom	54
Bartlett's Chi Square	270.759
P-Value	<.0001

### Eigenvalues

	Magnitude	Variance Prop.
Value 1	2.925	.293
Value 2	1.966	.197
Value 3	1.267	.127
Value 4	1.062	.106
Value 5	.782	.078

### PCA Axes

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
+ Startle	.193	.735	-.109	.331	.030
+ Movement	.231	.620	-.327	.274	.284
+ Contact (Lat)	.233	.629	.449	.161	.117
+ Total INV	-.075	-.119	.870	.044	.153
+ All Displays	.383	.352	.373	-.400	.025
+ Contact (Fight)	.880	-.264	-.024	.062	-.046
+ Insert (Fight)	.946	-.198	-.001	.068	-.117
+ 1st Rap	.938	-.156	-.005	.040	-.130
+ Raps/Bout	.106	-.579	-.016	.316	.686
+ Mean Pause	-.259	-.196	.224	.761	-.400

Table 6.1. PCA generating 4 factors with Eigen values > 1 from 10 behavioural variables.

**Table 6.2.** Correlations and paired *t*-tests for principal components factors between situations (low and high predation risk).

PCA result	Correlation Z-test			<i>t</i> -test			
	Z-Value	P-Value	<i>R</i>	<i>t</i> -Value	P-Value	Cohen's <i>d</i>	Effect size
PC1	1.953	0.0508	0.280	- 0.583	0.5627	- 0.008	- 0.004
PC2	1.987	<b>0.0469</b>	0.285	0.373	0.7109	0.017	0.009
PC3	1.328	0.1841	0.193	0.607	0.5468	0.085	0.042
PC4	- 0.364	0.7156	0.054	- 0.032	0.9747	0.053	0.027

In order to test the stability of these behavioural syndromes across the predation risk situations, correlation Z-tests and paired *t*-tests were carried out, and the results are included in Table 6.2 along with their effect size estimates (*R* for the correlations and Cohen's *d* and *r* values for the *t*-tests). There was a non-significant trend for a positive correlation between PC1 ( $Z = 1.953$ ,  $P = 0.0508$ ), and there was a significant correlation between the scores for the second component ( $Z = 2.399$ ,  $P = 0.0469$ ). This result is consistent with Briffa et al. (2008b) as well as the univariate result for consistent differences in startle responses. This is because a major factor influencing PC2 is startle response (see Table 6.1). There were no significant differences between the paired PCA factors across the two predation risk situations (see Table 6.2).

***Correlations between [Hc]<sub>HL</sub> and individual behaviours***

In order to test whether [Hc]<sub>HL</sub> is related to the behaviours observed, correlation Z-tests were carried out between each behaviour, and the [Hc]<sub>HL</sub>. Following Bonferroni corrections to the required *P*-values, no significant correlations were found between [Hc]<sub>HL</sub> and startle responses, or any of the behaviours associated with shell investigation or fighting in either situation (see Tables A.3 and A.4).

*Correlations between [Hc]<sub>HL</sub> and behavioural syndromes*

In order to test whether physiology by way of aerobic capacity is related to behavioural syndromes, correlation Z-tests were carried out between principle components identified, and the [Hc]<sub>HL</sub> (see Table 6.3).

**Table 6.3.** Correlations between [Hc]<sub>HL</sub> and behavioural syndromes

Variable	Low Risk			High Risk		
	Z-Value	P-Value	R	Z-Value	P-Value	R
PC1	1.031	0.3025	0.143	- 1.725	0.0846	- 0.225
PC2	0.287	0.7737	0.040	0.482	0.6297	0.064
PC3	- 0.296	0.7675	- 0.041	- 0.284	0.7766	- 0.038
PC4	- 0.225	0.8217	- 0.032	- 0.357	0.7215	- 0.047

There were no significant correlations between the [Hc]<sub>HL</sub> and any of the behavioural syndromes exhibited in the low predation risk situation. Similarly, in the high predation risk situation there were no significant correlations between [Hc]<sub>HL</sub> and PC2, PC3 or PC4 (see Table 6.3). However, a non-significant negative trend was found between the [Hc]<sub>HL</sub> and PC1 ( $Z = - 1.725$ ,  $P = 0.0846$ ).

*Effect of behavioural syndromes on fight outcomes*

In order to determine whether behavioural syndromes varied between fight outcomes (i.e. attacker evicts or fails to evict the defender), a series of one-factor ANOVAs was performed. The PCA scores were used as the dependent variables and the factor was fight outcome (eviction or non-eviction).

No significant difference was detected in any of the behavioural syndromes between the fight outcomes (see Table 6.4).

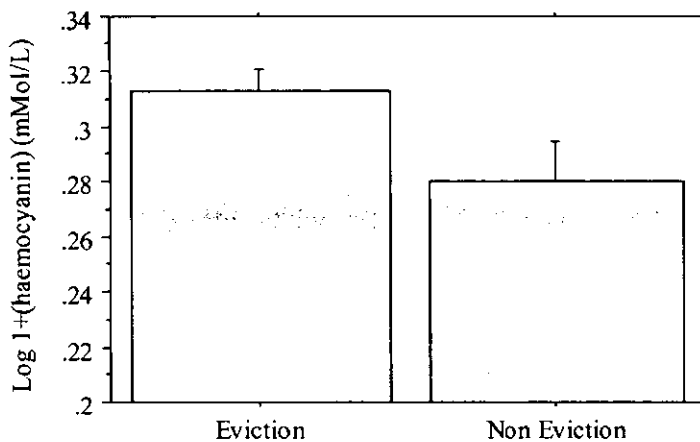


**Table 6.4.** ANOVA results for principal components factors between fight outcomes. The prefix (-) indicates a low risk or 'control treatment', while (+) indicates a high risk or 'predation cue treatment'.

PC Factor	DF	<i>F</i> - Value	<i>P</i> -Value	$\eta^2$
- PC1	51	0.076	0.7841	0.00149
- PC2	51	0.724	0.3989	0.01399
- PC3	51	0.060	0.8069	0.00119
- PC4	51	1.042	0.3122	0.02002
+ PC1	56	0.021	0.8854	0.00038
+ PC2	56	0.174	0.6784	0.00309
+ PC3	56	0.045	0.8329	0.00081
+ PC4	56	$4.928 \times 10^{-4}$	0.9824	$6.118 \times 10^{-6}$

### *Effect of [Hc]<sub>HL</sub> on fight outcomes*

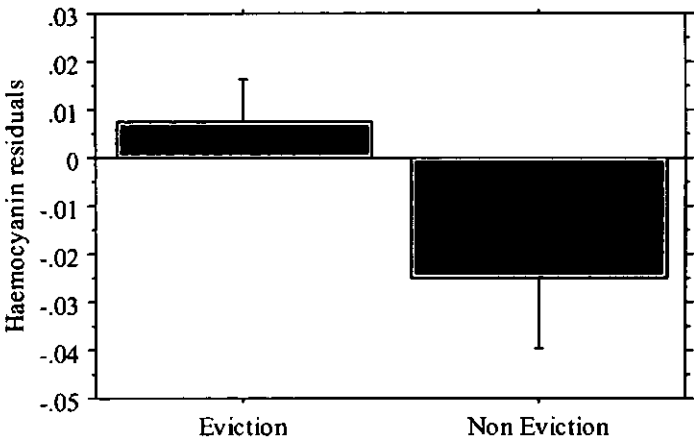
In order to investigate the effect of [Hc]<sub>HL</sub> on fight outcomes, a one-factor ANCOVA was performed. The [Hc]<sub>HL</sub> was the dependent variable in the model, while the weight of the crabs was the covariate. The factor included in the model was fight outcome (eviction or non-eviction). No significant interaction effect with the covariate was detected, and this was deleted from the model. There was a non-significant trend for higher [Hc]<sub>HL</sub> in winners of shell fights ( $F_{1,62} = 3.932$ ,  $P = 0.0518$ ,  $\eta^2 = 0.0612$ ; Figure 6.4).



**Figure 6.4.** [Hc]<sub>HL</sub> in relation to the outcome of the fight.

However, no significant relationship was detected between the  $[Hc]_{HL}$  and the weight of the crabs ( $F_{1,62} = 4.046E-4$ ,  $P = 0.9840$ ,  $\eta^2 = 6.12E-06$ ,  $R = 0.002$ ). Despite the loss of the positive relationship between  $[Hc]_{HL}$  and crab weight detected in Chapter 3, it is still possible to calculate residuals for the current  $[Hc]_{HL}$  by subtracting the mean logged  $[Hc]_{HL}$  value from each  $[Hc]_{HL}$  result.

In order to investigate the effect of residual  $[Hc]_{HL}$  on fight outcomes, a one-factor ANOVA was performed. The residual  $[Hc]_{HL}$  was the dependent variable in these models, and the factor included in the model was fight outcome (eviction or non-eviction). A significant difference was detected in the residual  $[Hc]_{HL}$  between the fight outcomes, where winners had positive residuals whereas losers had negative residuals ( $F_{1,63} = 4.056$ ,  $P = 0.0483$ ,  $\eta^2 = 0.0612$ ; Figure 6.5).



**Figure 6.5.**  $[Hc]_{HL}$  residuals in relation to the outcome of the fight.

## DISCUSSION

This study examined the boldness, exploratory and aggressive behaviour of hermit crabs for cross-situational correlations and differences. Furthermore, PCA was also used to determine whether these behaviours were correlated across different contexts, and the cross-situational stability of any behavioural syndromes found was examined. Finally, the effect of these syndromes on fight outcomes was investigated. Consistent individual differences were only demonstrated in one behaviour, the startle response duration, as was demonstrated by Briffa et al. (2008b). However the PCA revealed that a behavioural syndrome of correlated boldness and exploratory behaviours (PC2, Table 6.1) exists for *Pagurus bernhardus*, while there may also be a syndrome of correlated exploratory and aggressive behaviours (PC1, Table 6.1). PC1 coded for the initial fighting behaviours (latency to contact, cheliped insertion and rapping), the duration of pre-fight displays and the latency to contact the shell in the exploratory behaviour context, all of which were positively related to one another. However, the total time spent investigating the shell in the exploratory behaviour context, and the mean pause duration during shell fights were negatively correlated with these behaviours. PC2 coded for the duration of startle responses and the latency to explore, both of which were positively correlated (Table 6.1). However, in the low predation risk situation, these behaviours were negatively related with the duration of pre-fight displays, whereas in the high predation risk situation they were positively related. In addition, in the high predation risk situation, the number of raps/bout is negatively correlated with the startle responses, exploratory behaviours (latency to movement and latency to contacting the shell) and display duration whilst this trend is absent in the low risk situation. Despite this cross-situational variation, PC2 was found to be stable across situations while there was a strong non-significant trend for a positive relationship between situations for PC1 (see Table 6.2). Further, the effect size estimates indicate that the strength of consistency is stronger than the strength of flexibility for these syndromes between the situations (Table 6.2).

Haemolymph haemocyanin concentration was generally unrelated to the behaviours (Tables A.3 and A.4) or to the behavioural type (the configuration of behaviours expressed by an individual, Bell 2007) of the crabs (which was extracted from the PCA results; Table 6.3), although there was a non-significant negative trend between PC1 in the high predation risk situation and the  $[Hc]_{HL}$  of the crabs. Despite this, the  $[Hc]_{HL}$  played a role in influencing the outcome of fights whereas the behavioural type of the crabs did not. There were no differences in the behavioural type of the individual crabs between the fight outcomes (Table

6.4) whereas there was a strong non-significant trend for higher  $[Hc]_{HL}$  in the winners of shell fights. In contrast to the results of Chapter 3, these results indicate that the fighting ability of attackers is influenced by the  $[Hc]_{HL}$ . Also in contrast to Chapter 3, there was no positive relationship detected between  $[Hc]_{HL}$  and the weight of the crabs. However, residuals calculated from the actual  $[Hc]_{HL}$  of each crab relative to the predicted value (the mean  $[Hc]_{HL}$ ) demonstrate that again, those individuals that have higher  $[Hc]_{HL}$  than predicted are more likely to win shell fights, whereas those individuals that have lower  $[Hc]_{HL}$  than predicted are more likely to lose shell fights.

This study suggests the presence of animal personalities in *P. bernhardus* as evidenced by stable behavioural syndromes between situations. PC1 appears to correspond with behaviours on the proactive – reactive axis (Koolhaas et al. 1999), as the latency to fight is correlated with the duration of displays and the latency to contact. Thus, these individuals may adopt a strategy that involves gaining high levels of information before performing, but once engaged in an activity such as shell investigation or fighting, these individuals finish investigation more quickly and leave shorter pauses during shell fights. PC2 appears to demonstrate a shy – bold axis (Wilson et al. 1993) as individuals with shorter startle responses also take longer to initiate movement and shell investigation in the exploration context. However, despite the correlations between behaviours, the duration of pre-fight displays and the number of raps/bout are not stable between situations, indicating that a degree of context-specific plasticity is maintained.

Behavioural syndromes have not only been used to explain consistency, but also to explain apparently maladaptive traits. Indeed, many studies have shown that where behaviours are correlated across contexts, there may be negative consequences in terms of the individual's fitness (Duckworth 2006; Sinn et al. 2008). These come about where adaptive behaviours in one context lead to maladaptive behaviours in another context, often referred to as 'carryover' (Sinn et al. 2008) or 'spillover effects' (Chadwick Johnson & Sih 2007). For example, precopulatory sexual cannibalism in the fishing spider *Dolomedes triton* is correlated with boldness, and may either be a spillover from a juvenile feeding context (Arnqvist & Henriksson 1997) or from a suite of correlated contexts representing a broader syndrome (Chadwick Johnson & Sih 2005). This behaviour appears suboptimal as it is carried out without generating a mating and when it is not necessarily beneficial (Chadwick Johnson & Sih 2005). Similarly, a correlation between male-male aggression and nest defence in western bluebirds (*Sialia mexicana*) leads to reduced reproductive success as aggressive males defend their nests intensely and thus provide less parental care (Duckworth

2006). Thus, retaining plasticity in some contexts may be beneficial for fitness, and it appears that this may be occurring in *P. bernhardus* as a limited amount of plasticity in PC2 is evident between the situations (see Table 6.1).

Several studies suggest that the manifestation of behavioural syndromes may be affected by an animal's internal 'state', and that the most important aspect of an animal's state is its physiology (Dall et al. 2004). For example, consistent individual differences in risk-taking may be generated by differences in energetic reserves (Rands et al. 2003), leading to animals spending differing amounts of time in refugia, or foraging. Furthermore, behavioural syndromes may be affected by neuroendocrine mechanisms (for reviews see Bell 2007; Sih et al. 2004). Testosterone has been demonstrated to affect many aspects of behaviour in male birds, such as aggression, courtship and parental care (Ketterson & Nolan 1999). Similarly, it appears that two hormones may control both boldness and aggression in funnel web spiders *Agelenopsis aperta* (Riechert & Hedrick 1993). In this study, the  $[Hc]_{HL}$  was used as an index of state as it is a physiological correlate that is likely to strongly affect the behaviour of hermit crabs by influencing aerobic capacity, and has previously been shown to influence fighting ability (Chapter 3). However,  $[Hc]_{HL}$  was not correlated with any of the particular behavioural types exhibited by the individuals in the low predation risk situation, and only a non-significant negative trend was found between the  $[Hc]_{HL}$  and PC1 (proactive-reactive axis) in the high risk treatment (Table 6.3). Thus, the 'state' of the hermit crabs in terms of their aerobic potential does not appear to influence their particular behavioural type on the axis. It is possible that  $[Hc]_{HL}$  was not the best substance to analyse a physiological effect on animal personality. Perhaps a better mechanism to study this would be the concentration of magnesium ions in the haemolymph ( $[Mg^{2+}]_{HL}$ ) as these ions are known to affect aerobic capacity (Nies et al. 1992; Terwilliger 1998; Truchot & Lallier 1992) as well as activity levels (for a review see Morritt & Spicer 1993) and aggression (Holliday 1980). In addition, hormones are also known to influence behavioural syndromes (Ketterson & Nolan 1999; Riechert & Hedrick 1993) as well as agonistic behaviour in crustaceans (monoamines, Briffa & Elwood 2007; Sneddon et al. 2000b) and thus may play a role in the development of animal personalities. However, it is difficult to speculate which proximate aspects of an animal may be of the most importance in influencing behaviour as many morphological and physiological factors act in combination (see Chapter 4).

Although hermit crabs were found to have consistent individual differences in behaviour, the measures of behavioural syndromes (behavioural types) had no effect on the outcome of shell fights (Table 6.4). This is in contrast to several studies that have found behavioural

syndromes to have links to individual fitness (e.g. Dingemanse et al. 2004; Réale et al. 2000). Perhaps there is another behavioural aspect to these syndromes that affects fitness, such as courtship and mating success, thus leading to their evolution. Alternatively, the costs of plasticity alone may be sufficiently high to generate these syndromes. Further, it appears that the capacity to win shell fights rests not with behavioural type, but with the physical properties of the individual. Although  $[Hc]_{HL}$  did not affect behavioural type, there was a strong non-significant trend for higher  $[Hc]_{HL}$  in attackers that evicted defenders than in attackers that failed to effect evictions (Figure 6.4). This is in agreement with the general results of Chapter 3, although in Chapter 3 there was no difference in the  $[Hc]_{HL}$  between the outcomes for attackers. This difference may be an effect of season as these studies were carried out at different times of the year (Chapter 3 = December 2006 – March 2007, Chapter 6 = April 2008 – July 2008), and  $[Hc]_{HL}$  may vary seasonally in response to temperature and food availability (Hagerman 1986; Redmond 1955). Furthermore, the original positive relationship between crab weight and  $[Hc]_{HL}$  (Figure 3.2) was not found in this study. In the absence of this trend,  $[Hc]_{HL}$  residuals were this time calculated as deviation from the mean (predicted) concentration. Again, a significant difference was detected in the  $[Hc]_{HL}$  residuals between the fight outcomes. Successful attackers that evicted defenders had positive residuals whereas unsuccessful attackers that failed to evict defenders had negative residuals (Figure 6.5). Similarly to the results for raw  $[Hc]_{HL}$ , this trend was not found in the December – March attackers analysed in Chapter 3. These results indicate that the respiratory physiology of hermit crabs imposes a greater constraint on the ability to win fights than does their ‘personality’.

During hermit crab shell fights, the crabs adopt two very different roles: attacker and defender. These perform distinctly different activities during the course of the encounter. The attacking crab initiates the fight, and performs the most obviously demanding behaviour by engaging in shell rapping, while the defender remains tightly withdrawn inside its gastropod shell. Shell rapping is a repeated signal suggested to advertise the attacker’s RHP by demonstrating its stamina, and it has been consistently shown that attackers that rap more vigorously are more likely to win shell fights (Briffa et al. 1998). Further, a set of related studies have demonstrated that the winners of shell fights are better able to withstand the physiological consequences of strenuous activity (Briffa & Elwood 2001; Briffa & Elwood 2002; Briffa & Elwood 2004; Briffa & Elwood 2005), and that winners have higher levels of stamina (Chapter 4). Thus, it is not surprising that  $[Hc]_{HL}$  affects fighting ability while behavioural syndromes do not. Even though the behavioural type of an animal may influence

its motivation, and propensity to engage in fights, it appears that the ability of the individual is a far more important aspect of RHP than underlying personality traits such as 'boldness', 'inquisitiveness' or 'aggressiveness'. For example, Sneddon et al. (2000) found that claw strength influenced the ability of shore crabs (*Carcinus maenas*) to win fights, but did not influence the propensity of crabs to initiate fights.

Animal personalities in terms of consistent boldness between situations have previously been demonstrated for *P. bernhardus*. The present results now demonstrate the existence of cross-contextual correlated suites of behaviour. Furthermore, these 'behavioural syndromes' appear to be stable across situations, indicating that behavioural types in hermit crabs are not restricted to one behavioural context such as boldness. Nevertheless, while it was predicted that, as in other examples (e.g. fiddler crabs, Reaney & Backwell 2007), these behavioural syndromes might influence fighting ability, this does not appear to be true of hermit crabs. Instead, individual capacities for performing demanding agonistic behaviours appear to be the key determinant of agonistic success. Thus, while there may be individual differences in behaviour, or 'personality', it is the differences in performance which affect the ability to win fights.

# CHAPTER 7

## Discussion



## **Summary**

The aim of this thesis was to investigate the proximate physiological and whole-body correlates of contest behaviour using shell fights in the hermit crab *Pagurus bernhardus* as a model system. It aimed to highlight understudied mechanisms that may govern contest behaviour, and to investigate components that may help our understanding of agonistic signalling. The correlates of fighting ability measured ranged from fundamentally small factors to holistic whole organism measures, demonstrating that individual factors or multiple components may affect the fighting ability of animals. The first factor to be analysed was the affect of metal ions on fighting ability. After the finding that heightened  $Mg^{2+}$  and  $Ca^{2+}$  may enhance aerobic capacity, the concentration of the crustacean respiratory pigment haemocyanin ( $[Hc]_{HL}$ ) itself was measured. In order to test the hypothesis that an individual's stamina may affect its fighting ability, the performance capacities of contestants were measured by making the hermit crabs run around a circular raceway. In the absence of a non-injurious measure of strength, the quality of the abdominal muscles of the opponents was investigated as a surrogate measure, analysing relative muscle size and protein concentration. Finally, another whole organism measure was taken, but focussing on 'personality' rather than performance. Behaviours were analysed for cross-context correlations and the syndromes revealed were tested for relatedness to fighting behaviour. In addition, a measure of physiological 'state' was taken using  $[Hc]_{HL}$ , which was also tested for relatedness to the syndromes found and fighting ability. Thus, the correlates of contest behaviour covered by this thesis span from simple ions through more complex structures including proteins, to muscle groups, then physical whole animal measures and finally to abstract whole animal behavioural measures termed 'personality'.

## **General findings**

The fighting ability of *P. bernhardus* was found to be correlated with physiological (Chapters 2, 3 and 5), morphological (Chapter 5) and whole organism factors by way of performance (Chapter 4), but not 'personality' (Chapter 6). The initial study focussed on the role that metal ions play in influencing agonistic behaviour (Chapter 2). The rationale for this study was that higher levels of  $Mg^{2+}$  could have negative consequences for fighting ability through a narcotising effect by interfering with neuro-muscular function (see Boardman & Collier 1946; Purves et al. 2001). The apparently counterintuitive finding that both  $Mg^{2+}$ , and its

antagonist ion, calcium ( $\text{Ca}^{2+}$ ) increased the fighting ability of hermit crabs led to the new hypothesis that elevated concentrations of these ions may increase fighting ability by enhancing the oxygen affinity of haemocyanin (Hc), thereby increasing the aerobic capacity of the crabs (Nies et al. 1992; Terwilliger 1998; Truchot & Lallier 1992). The narcotising effects of  $\text{Mg}^{2+}$  would still be present, but may feature in a trade-off whereby the current scope for activity is slightly reduced in favour for increasing aerobic capacity and delaying the consequences of anaerobic respiration. As  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  can be reabsorbed from the exoskeleton, this process would also liberate carbonate ions, which may further help combat anaerobic stress by buffering the increase in haemolymph *L*-lactate (Cameron 1985; DeFur et al. 1980; Henry et al. 1981).

As the oxygen affinity of Hc appears to be important to fighting hermit crabs, it is likely that the concentration of this respiratory pigment may be important as well. In addition, it has been found in previous studies that the concentration of respiratory pigment affects the degree of activity that a crustacean can undertake (Kobayashi & Gonoï 1985). Thus, the second experiment investigated the effects of haemocyanin concentration on hermit crab shell fights (Chapter 3). It was found that the winners of shell fights (attackers that evicted defenders, and defenders that resisted eviction) had higher  $[\text{Hc}]_{\text{HL}}$  than losers (attackers that failed to evict defenders, and defenders that were evicted). Furthermore,  $[\text{Hc}]_{\text{HL}}$  was found to be positively correlated with the weight of the crabs, and residuals calculated from this relationship revealed that the winners of shell fights had higher  $[\text{Hc}]_{\text{HL}}$  than predicted for their size whereas the losers of shell fights had lower  $[\text{Hc}]_{\text{HL}}$  than predicted for their size.

As aerobic capacity is likely to be closely linked to stamina (the capacity to perform a behaviour at a specific rate over a prolonged period), the effects of performance capacities (Huyghe et al. 2005; Perry et al. 2004) on hermit crab shell fights were measured (Chapter 4). The winners of shell fights were found to have higher mean and top speeds than the losers of shell fights, indicating for the first time that stamina affects the fighting ability of hermit crabs. Another aspect of performance capacity which can be important to fighting animals is strength (Huyghe et al. 2005; Lailvaux et al. 2004; Sneddon et al. 2000a). In the absence of a non-injurious method of recording the strength of a hermit crab, the muscle quality of hermit crabs was used as a surrogate measure (Chapter 5). It was found that muscle protein concentration did not vary between the winners and losers of shell fights, although the muscle protein concentration of defenders appears to be correlated with the mean pause duration of the pattern of shell rapping that they receive. However, there was a non-significant trend for higher overall muscle/body weight ratios in the winners of shell fights, and defenders that

resisted eviction had significantly higher muscle/body weight ratios than defenders that were evicted. Thus, both stamina and strength appear to be important to fighting hermit crabs.

The final investigation (Chapter 6) examined how another whole animal measure may affect aggression and fighting ability. However, rather than investigating a physical performance-based measure, this experiment explored the possibility of ‘personality’ in *P. bernhardus*, and whether either ‘behavioural syndromes’ (Dall et al. 2004; Sih et al. 2004) or an individual’s ‘state’ (see Dall et al. 2004) affected fighting ability, or each other. The presence of two behavioural syndromes was revealed, which were estimated to represent both a ‘proactive – reactive axis’ (Koolhaas et al. 1999) and a ‘shy – bold axis’ (Wilson et al. 1993). These syndromes were not related to an individual’s ‘state’, as measured by [Hc]<sub>HL</sub>, or to the fighting ability of attackers. However, there was a strong non-significant trend for higher [Hc]<sub>HL</sub> in attackers that effected evictions, and residuals calculated from the average [Hc]<sub>HL</sub> indicated that attackers that won had significantly higher [Hc]<sub>HL</sub> than predicted whereas attackers that lost had lower [Hc]<sub>HL</sub> than predicted. Thus, this final investigation revealed the presence of ‘personality’ in the hermit crab *P. bernhardus*, but that despite an individual’s behavioural type; it is its performance capabilities that dictate whether it will succeed in fights.

### *Aerobic capacity and stamina*

One of the main themes of this thesis has been the aerobic capacity of opponents, and how this influences stamina. Repeated signals such as the shell rapping of hermit crabs have been previously demonstrated to be related to physiological correlates of stamina, such as the accumulation of metabolic by-products (e.g. *L*-lactate, Briffa & Elwood 2001; Briffa & Elwood 2004; Briffa & Elwood 2005) and the depletion of energy reserves (e.g. glycogen, Briffa & Elwood 2001; Briffa & Elwood 2004). The physiological mechanisms contributing to stamina are thus the energy reserves, and the respiratory capacity available to an individual. In turn, respiration occurs through both aerobic and anaerobic pathways, although anaerobic respiration is more costly than aerobic respiration (Sadava et al. 2008). Challenging and exceeding aerobic capacity leads to anaerobic respiration, which is less efficient as it liberates less ATP per molecule of glucose (Sadava et al. 2008) and results in the accumulation of *L*-lactate (for reviews see Bridges 2001; Briffa & Sneddon 2007). The concentrations of this metabolite can thus be used to demonstrate that demanding activity has

been undertaken as the animals have undergone anaerobic respiration (e.g. Briffa & Elwood 2005).

Chapter 3 of this thesis examined the fighting ability of hermit crabs in relation to their baseline aerobic capacity in terms of their  $[Hc]_{HL}$ . This gave an indication of their aerobic capacity, rather than challenging them and then observing the resulting physiological consequences. Similar to the results of Chapter 2, it was demonstrated that individuals with higher aerobic capacity were more likely to win shell fights, although unlike the metal ions analysed in Chapter 2,  $[Hc]_{HL}$  is likely to remain stable throughout the course of contests. Thus, both dynamic and fixed factors influence aerobic capacity, and are therefore likely to affect the stamina of contestants. However, these measures are again only correlates of stamina, and do not represent stamina *per se*. Chapter 4 tested the stamina (the capacity to perform a behaviour at a specific rate over a prolonged period) of hermit crabs by measuring their mean and top speeds over 15 m along a circular raceway, revealing that individuals with greater performance capacities were better competitors in hermit crab shell fights. Thus, stamina itself was shown to determine fighting ability, in agreement with models such as the energetic war of attrition model (E-WOA: Payne & Pagel 1996; Payne & Pagel 1997) and the cumulative assessment model (CAM: Payne 1998).

### ***The importance of performance capacities***

The performance capacity experiment presented in Chapter 4 allowed the actual quantification of the stamina of the contestants via the performance of an ‘ecologically relevant task’ (Lailvaux & Irschick 2007), in this case, running. In addition, experiments into performance capacity take into account all of the physiological, morphological and behavioural aspects of an animal’s phenotype, and thus present the observer with an overall measure of the individual’s ability. These combined factors should be the primary targets of selection (Lailvaux et al. 2004), and may thus correlate with Darwinian fitness (for a review see Irschick & Garland Jr 2001).

The results of Chapter 4 revealed that the winners of shell fights had greater performance capacities than losers. Furthermore, when the effect size estimates ( $\eta^2$ ) from this experiment were compared with those from the haemocyanin experiment (Chapter 3), the  $\eta^2$  for performance capacities were found to be larger. In addition to the smaller *P*-values generated by the performance capacity experiment, this indicates that performance capacities are a

better predictor of fighting ability than  $[Hc]_{HL}$  alone. Thus, the overall measures of competitors are likely to be more important in determining fighting ability than any one physiological, morphological or behavioural variable in isolation.

### ***The importance of personality***

Similar to experiments into performance capacity, the investigation of ‘behavioural syndromes’, or ‘animal personality’ is a holistic method, seemingly returning to the original ethological perspective of studying animal behaviour. As with performance capacities, where all of the components of an animal’s phenotype determine what level of activity it is capable of undertaking, from the perspective of animal personalities all of the correlated behaviours across contexts may determine how an animal will behave in an additional context. For example, it was suggested that unnecessary precopulatory sexual cannibalism in the fishing spider *Dolomedes triton* was a ‘spillover’ (Chadwick Johnson & Sih 2005) of behaviour from either the juvenile feeding context (Arnqvist & Henriksson 1997) or from a suite of correlated contexts representing a broader syndrome (Chadwick Johnson & Sih 2005). Thus, it may be necessary to examine behaviours from several contexts in order to understand fully why a certain behaviour is performed in another context (Chadwick Johnson & Sih 2005).

### ***Behavioural syndromes in hermit crabs***

The investigation undertaken in Chapter 6 revealed consistent individual differences in the startle responses of hermit crabs, similar to the findings of Briffa et al. (2008b), although there were no cross-situational correlations between any of the other individual behaviours analysed. However, the experiment did reveal the existence of behavioural syndromes (correlated suites of behaviours) in hermit crabs, incorporating behaviours correlated across the contexts of boldness, exploration and aggression. Further, the two factors identified by the PCA appear to be consistent with the ‘proactive – reactive’ (Koolhaas et al. 1999) and ‘shy – bold’ axes (Wilson et al. 1993).

Behavioural syndromes are still a relatively recent development in the study of animal behaviour, but may contribute towards understanding the ‘limited plasticity’ identified by Hazlett (1995). As with many behavioural questions, studies of behavioural syndromes tend to focus on vertebrates such as mammals (e.g. Réale et al. 2000), birds e.g. (Duckworth

2006), amphibians e.g. (Sih et al. 2003) and fish e.g. (e.g. Sneddon 2003; Wilson 1998; Wilson et al. 1993), although studies have shown that behavioural syndromes also occur in invertebrates such as spiders (Chadwick Johnson & Sih 2005), fiddler crabs (Reaney & Backwell 2007), hermit crabs (Briffa et al. 2008b) and squid (Sinn et al. 2008).

The study presented in Chapter 6 expands on the work presented by Briffa et al. (2008b), demonstrating that the behaviours of hermit crabs are not only correlated temporally, but also across contexts. However, the behavioural type of individuals did not affect their fighting ability, and was not related to their 'state' in terms of  $[Hc]_{HL}$ , although there was a non-significant trend between PC1 in the high predation risk situation and the  $[Hc]_{HL}$  of the crabs (Chapter 6, Table 3). Perhaps 'state' does not influence the behavioural syndromes of hermit crabs, or perhaps  $[Hc]_{HL}$  is not the most appropriate measure of 'state' for this system. It is possible that energy reserves (Briffa & Elwood 2004; Rands et al. 2003), hormones (Ketterson & Nolan 1999; Riechert & Hedrick 1993) or  $[Mg^{2+}]_{HL}$  are more appropriate measures.

Although similar to haemocyanin, in that elevated  $[Mg^{2+}]_{HL}$  increases aerobic capacity (Nies et al. 1992; Terwilliger 1998; Truchot & Lallier 1992),  $[Mg^{2+}]_{HL}$  is also known to affect both levels of aggression (Holliday 1980), and activity (for a review see Morritt & Spicer 1993) within-species (Spicer et al. 1994) and across taxa (Walters & Uglow 1981). As suggested by Sih et al. (2004), behavioural syndromes may occur either within species or across groups of species, where each species within the group has its own behavioural type. This may be true of British species of Brachyuran crabs, and may be mediated by 'state' in terms of  $[Mg^{2+}]_{HL}$ . For example, the highly aggressive and active *Necora puber* and other Portunids have the lowest average  $[Mg^{2+}]_{HL}$  of the common British brachyuran crabs, whereas the relatively passive and slow *Maja squinado* has very high  $[Mg^{2+}]_{HL}$  (see Walters & Uglow 1981). Thus, there may be a behavioural syndrome across crustacean taxa, whereby aggression and activity are linked to  $[Mg^{2+}]_{HL}$  along a shy – bold continuum, although this has yet to be explored.

### ***The two roles of shell - fighting hermit crabs***

In hermit crab shell fights, the opponents adopt two very different roles: attacker and defender. The attacking crab performs the most obviously demanding activity by engaging in shell rapping, during which the defender remains tightly withdrawn inside its shell. Because

of this, the two separate roles face differing physiological constraints during the contest, and it has previously been shown that attackers decide to withdraw from shell fights when the amount of *L*-lactate generated crosses a threshold level (Briffa & Elwood 2001; Briffa & Elwood 2004; Briffa & Elwood 2005), whereas the defender's decision to withdraw is based on a failure to mobilise energy reserves (Briffa & Elwood 2001; Briffa & Elwood 2004).

The experiments that make up this thesis revealed a number of other differences between the decisions, physiology and performance of the two roles. Firstly, although there were interactions demonstrating that the winners of shell fights had both higher  $[Mg^{2+}]_{HL}$  and  $[Ca^{2+}]_{HL}$  than losers, when the roles were analysed separately, there was only a non-significant trend for higher  $[Ca^{2+}]_{HL}$  in attackers that won, and no differences between the outcomes for defenders (Chapter 2). In contrast, in Chapter 3, interactions demonstrated that the winners of shell fights had higher  $[Hc]_{HL}$  and positive Hc residuals, whereas losers had lower  $[Hc]_{HL}$  and negative Hc residuals. However, when the roles were analysed separately it was found that there were no differences between the outcomes for attackers whereas these differences were significant for defenders. Thus, it appears that the two roles rely on different mechanisms to boost their aerobic capacity during shell fights. Attackers utilise metal ions to a greater extent than defenders, possibly as they are free to exchange ions with the surrounding water whereas the amount of water available to defenders may be limited within the confines of the shell. Crustaceans may draw ions from both the environment and their calcified exoskeleton (DeFur et al. 1980), although environmental ions are only available to aquatic crustaceans, and terrestrial species tend to utilise their integument (Cameron 1985). Thus, it is likely that attackers have greater access to ions than defenders as they are able to alter ionic regulation using the surrounding seawater, without having to reabsorb ions from their exoskeleton as much. Because of this, defenders may have to rely on baseline haemocyanin levels in order to obtain enough oxygen to persist in shell fights, leading to the differences between the roles.

In Chapter 4, the aspects of performance capacity that were important to fighting ability were also found to differ between the roles adopted by the crabs. Stamina (measured as mean speed) was demonstrated to be higher in both attackers and defenders that won shell fights, than in those that lost. However, although an interaction demonstrated that the top speeds of the crabs were higher in the winners of shell fights, when the roles were analysed separately, this was only true for defenders, with no difference between the outcomes for attackers. This may illustrate the difference between maximum endurance and maximum effort. As attackers appear to perform shell rapping in order to demonstrate their stamina (Briffa & Elwood

2000a), it is likely that maximum endurance is of most importance to this role. Similarly, defenders must resist the shell rapping and the attempts at eviction by attackers for the duration of the shell fight. Furthermore, as they remain withdrawn inside their contested shells for the duration of the encounter, it is likely that the levels of available oxygen will fall. Thus, defenders that win have higher levels of haemocyanin, but also higher maximum endurance as well. Therefore maximum endurance is important to both roles. However, maximum effort appears to be of importance to defenders only. It is likely that this is because defenders have to hold onto their shell during key moments of the encounter where attackers attempt to evict them.

Although maximum effort may be more important in determining the fighting ability of defenders rather than attackers, it appears that strength may be more important to attackers than defenders. The surrogate measure of muscle/body weight ratio taken in Chapter 5 indicated that the winners of shell fights had larger abdominal muscles relative to their whole body weight than losers. However, separate analyses of each role revealed that only defenders that won had significantly higher muscle/body weight ratios than those that lost. It is likely that relatively larger muscles allow defenders to hold on more strongly to the contested shell.

Overall, the studies within this thesis have revealed how many different factors can affect the fighting ability of hermit crabs, although it appears that all of them can affect the different roles in different ways.

### ***The importance of seasonality***

In addition to differences in the physiological correlates between the roles and outcomes of the hermit crabs, some temporal differences consistent with seasons were also revealed. In Chapter 2, the  $[Mg^{2+}]_{HL}$  decreased between spring and summer as the seasons became warmer, while the  $[Ca^{2+}]_{HL}$  increased. These changes varied between the roles adopted by the crabs and also influenced their fighting ability. It is likely that seasonal changes in temperature affect the ability of hermit crabs to regulate metal ions (Spicer et al. 1994), and those individuals that are better ionic regulators may be better competitors during shell fights.

A temporal change in  $[Hc]_{HL}$  was also found between experiments. In Chapter 3,  $[Hc]_{HL}$  was not significantly different between the outcomes for attackers, and it was positively correlated with crab weight. However, in Chapter 6, there was a strong non-significant trend



for higher haemocyanin in attackers that won, while the relationship with crab weight had been lost. Residuals calculated instead from the average  $[Hc]_{HL}$  revealed a significant difference between the outcomes for attackers, where attackers that effected evictions had positive residuals while those that did not effect evictions had negative residuals. Again, this difference was not present in Chapter 3. It is likely that this is an effect of season as these studies were carried out at different times of the year, with Chapter 2 being conducted in the winter (December 2006 – March 2007) while Chapter 5 was conducted in the early summer (April 2008 – July 2008). The  $[Hc]_{HL}$  may have changed in response to temperature or food availability (Hagerman 1986; Redmond 1955), leading to the changes observed in the fighting ability of the attackers.

These results demonstrate that seasonal changes can affect fighting ability in animals that have to fight over resources throughout the year. These changes are thus not restricted to annual cycles in behaviour and physiology associated with contests over temporally isolated resources such as mates during the breeding season, for example the rut in red deer, *Cervus elaphus* (see Clutton-Brock & Albon 1979; Clutton-Brock et al. 1979 and references therein). Furthermore, seasonal changes mean that future studies of behaviour may have to take season into account when there are temporal gaps in data, and conduct investigations either as within-season blocks to avoid such factors, or throughout the whole year in order to include the effects of season.

### ***Further work***

Shell fights in the hermit crab *P. bernhardus* have proved to be an extremely tractable model system for the study of the physiological and whole-organism correlates of contest behaviour. The experiments presented in this thesis have generated further questions which may expand upon the conclusions presented here. First, as it has been shown that higher  $[Hc]_{HL}$  is beneficial for fighting hermit crabs, it would be interesting to manipulate  $[Hc]_{HL}$  prior to staging fights in order to determine whether  $[Hc]_{HL}$  as a causal factor. This is possible by maintaining hermit crabs at reduced oxygen tensions (e.g. 40%  $O_2$  saturation or 8kPa) (DeFur et al. 1990; Hagerman 1986; Spicer & Baden 2001) for 24 hours. It would also be worthwhile to measure  $[Hc]_{HL}$  as well as the metal ions  $[Mg^{2+}]_{HL}$  and  $[Ca^{2+}]_{HL}$  in relation to performance capacity in order to test whether that these physiological variables are correlates of stamina as well as fighting ability.

As it has been shown in Chapter 4 that attackers and defenders may rely on different aspects of performance capacity in order to win shell fights, it would be interesting to establish whether the roles do rely on endurance or burst activity by analysing the ratios of fast and slow twitch fibres in dissected abdominal muscles. It would be expected that defenders that won would have slightly more fast than slow twitch fibres than defenders that lost as they appear to rely on both maximum endurance, but maximum effort as well in order to hold onto their shell. Attackers that won would be predicted to have more slow than fast twitch fibres than attackers that lost, as attackers rely on maximum effort to win shell fights by performing a signal of stamina.

As it has been shown in Chapter 6 that *P. bernhardus* exhibits behavioural syndromes, it would be interesting to see whether additional contexts are involved, such as behaviours associated with breeding (e.g. Chadwick Johnson & Sih 2005; Reaney & Backwell 2007). Furthermore, it would be interesting to explore which aspects of physiological 'state' may affect the 'personality' of hermit crabs, as well as examining the effects of variables such as  $[Mg^{2+}]_{HL}$  across crustacean taxa.

Lastly, as  $[Ca^{2+}]_{HL}$ ,  $[Mg^{2+}]_{HL}$  and  $[Hc]_{HL}$  have been demonstrated to be influenced by season, it would be of interest to carry out a long-term experiment tracking both the levels of these physiological variables and that of hermit crab RHP across the seasons for one annual rotation.

## Conclusions

The work presented in this thesis demonstrates that a suite of physiological and whole-organism variables influence contest behaviour in hermit crabs. Aerobic capacity is very important as it confers a greater scope for activity before the onset of anaerobic respiration, and therefore contributes to stamina, which is assumed by theory to be important during agonistic signalling (e.g. the E-WOA: Payne & Pagel 1996; Payne & Pagel 1997). Higher aerobic capacities are found in the winners of shell fights, and this may be achieved by elevating the  $[Mg^{2+}]_{HL}$  and  $[Ca^{2+}]_{HL}$ , or by having higher levels of  $[Hc]_{HL}$ , although which mechanism is of benefit is dependent on the role adopted by the crabs.

Increased aerobic capacity contributes to stamina, and it has been shown here for the first time that there is a direct link between the stamina of hermit crabs and their fighting ability. Furthermore, as the effect size estimates for the performance capacity experiment were

greater than those for the haemocyanin experiment, it can be concluded that performance capacities are better predictors of fighting ability than any one physiological or morphological aspect of an animal's phenotype in isolation. Despite this, it has also been demonstrated that although whole organism factors are important determinants of fighting ability, this is only the case in terms of performance, not personality.

By investigating whole-organism factors it was shown that performance capacities were important to fighting ability, and also that *P. bernhardus* exhibited behavioural syndromes consistent with 'personality'. This is the first time that a suite of correlated behaviours have been demonstrated in a hermit crab, adding weight to the argument that non-vertebrate animals can possess 'personality'. The use of a small invertebrate has provided an extremely tractable model system, allowing large numbers of individuals to be used. Furthermore, the propensity of *P. bernhardus* to engage in contests, along with the ease with which physiological samples can be obtained make it an effective system to study the biology of fighting. The present results and suggested future directions indicate that *P. bernhardus* remains a useful model system for the study of the physiological and whole-organism correlates of contest behaviour, as well as the expression of behavioural syndromes in invertebrates.

## APPENDIX

Univariate analyses carried out to test for correlations (Table 6.1) and differences (Table 6.2) between the low risk and high risk treatments for all of the behaviours recorded in Chapter 6.

**Table A.1.** Correlations for individual behaviours between predation risk situations. Required P2 indicates the Bonferroni corrected significance level.

Variable	Z-Value	P-Value	R	Required P2	Significance
<b>Boldness</b>					
Startle duration	7.752	< 0.0001	0.748	<b>0.00238</b>	<b>S</b>
<b>Exploration</b>					
Latency to move	0.356	0.7217	0.044	0.01250	NS
Latency to contact	0.595	0.5516	0.078	0.00833	NS
Latency to rotate	- 0.196	0.8444	- 0.028	0.02500	NS
Latency to insert chelipeds	- 0.138	0.8905	- 0.020	0.05000	NS
Contact duration	0.229	0.8190	0.030	0.01667	NS
Rotation duration	0.642	0.5206	0.092	0.00556	NS
Cheliped insertion duration	2.344	0.0191	0.329	0.00250	NS
Total investigation duration	0.956	0.3390	0.119	0.00455	NS
<b>Aggression</b>					
Presentation duration	0.793	0.4276	0.099	0.00500	NS
Extension duration	0.561	0.5751	0.070	0.01000	NS
Ambulatory raise duration	2.204	0.0275	0.269	0.00263	NS
High posture duration	0.622	0.5336	0.078	0.00625	NS
Total cheliped displays	0.614	0.5390	0.077	0.00714	NS
Total walking leg displays	1.054	0.2917	0.131	0.00385	NS
Total displays duration	1.013	0.3111	0.126	0.00417	NS
Latency to contact defender	1.336	0.1815	0.167	0.00357	NS
Latency to insert chelipeds	1.513	0.1304	0.188	0.00313	NS
Latency to first rap	1.875	0.0608	0.237	0.00294	NS
Raps/bout	1.367	0.1716	0.175	0.00333	NS
Mean pause duration	1.889	0.0589	0.258	0.00278	NS

**Table A.2.** Paired *t*-tests for individual behaviours between predation risk situations. Required P2 indicates the Bonferroni corrected significance level.

Variable	<i>t</i> -Value	<i>P</i> -Value	Cohen's <i>d</i>	Effect size <i>r</i>	Required <i>P</i> 2	Significance
<b>Boldness</b>						
Startle duration	0.865	0.3900	0.076	0.038	0.00333	NS
<b>Exploration</b>						
Latency to move	1.469	0.1465	0.247	0.122	0.00263	NS
Latency to contact	0.023	0.9820	0.002	0.001	0.05000	NS
Latency to rotate	- 1.000	0.3219	0.178	0.089	0.00313	NS
Latency to insert chelipeds	0.092	0.9274	0.101	0.051	0.01250	NS
Contact duration	2.534	0.0139	0.432	0.211	0.00250	NS
Rotation duration	2.615	0.0117	0.549	0.265	0.00238	NS
Cheliped insertion duration	0.098	0.9223	0.138	0.069	0.01000	NS
Total investigation duration	0.290	0.7724	0.048	0.024	0.00714	NS
<b>Aggression</b>						
Presentation duration	1.022	0.3104	0.152	0.076	0.00294	NS
Extension duration	- 1.096	0.2772	-0.2	-0.099	0.00278	NS
Ambulatory raise duration	- 0.602	0.5492	-0.086	-0.043	0.00385	NS
High posture duration	-0.081	0.9357	0	0.9357	0.01667	NS
Total cheliped displays	0.540	0.5912	0.09	0	0.00417	NS
Total walking leg displays	- 0.345	0.7310	-0.08	-0.04	0.00556	NS
Total displays duration	0.353	0.7249	0.054	0.027	0.00500	NS
Latency to contact defender	- 0.301	0.7646	-0.067	-0.034	0.00625	NS
Latency to insert chelipeds	- 0.230	0.8191	-0.055	-0.027	0.00833	NS
Latency to first rap	- 0.690	0.4925	-0.139	-0.069	0.00357	NS
Raps/bout	0.376	0.7079	0.069	0.035	0.00455	NS
Mean pause duration	0.042	0.9668	0.057	0.029	0.02500	NS

Univariate analyses carried out to test for correlations between [Hc]<sub>HL</sub> and individual behaviours performed in the low predation risk situation (Table 6.3) and in the high predation risk situation (Table 6.4) in Chapter 6.

**Table A.3.** Correlations between [Hc]<sub>HL</sub> and individual behaviours in the low predation risk situation

Variable	Z-Value	P-Value	R	Required P2	Significance
<b>Boldness</b>					
Startle duration	0.725	0.4684	0.090	0.00313	NS
<b>Exploration</b>					
Latency to move	- 0.303	0.7618	- 0.038	0.00625	NS
Latency to contact	- 0.083	0.9338	- 0.011	0.02500	NS
Latency to rotate	0.170	0.8648	0.023	0.01000	NS
Latency to insert chelipeds	0.344	0.7309	0.047	0.00556	NS
Contact duration	- 0.722	0.4701	- 0.094	0.00333	NS
Rotation duration	- 0.887	0.3749	- 0.120	0.00294	NS
Cheliped insertion duration	0.451	0.6523	0.062	0.00385	NS
Total investigation duration	0.405	0.6856	0.051	0.00417	NS
<b>Aggression</b>					
Presentation duration	- 0.947	0.3437	- 0.118	0.00278	NS
Extension duration	- 1.635	0.1020	- 0.202	0.00238	NS
Ambulatory raise duration	0.234	0.8146	0.029	0.00833	NS
High posture duration	0.033	0.9739	0.004	0.05000	NS
Total cheliped displays	- 1.281	0.2002	- 0.159	0.00250	NS
Total walking leg displays	0.156	0.8758	0.020	0.01250	NS
Total displays duration	- 0.972	0.3309	- 0.121	0.00263	NS
Latency to contact defender	- 0.390	0.6964	- 0.049	0.00455	NS
Latency to insert chelipeds	- 0.704	0.4811	- 0.089	0.00357	NS
Latency to first rap	- 0.116	0.9077	- 0.015	0.01667	NS
Raps/bout	0.369	0.7119	0.047	0.00500	NS
Mean pause duration	0.252	0.8009	0.034	0.00714	NS

**Table A.4.** Correlations between [Hc]<sub>HL</sub> and individual behaviours in the high predation risk situation

Variable	Z-Value	P-Value	R	Required P2	Significance
<b>Boldness</b>					
Startle duration	0.390	0.6964	0.049	0.01000	NS
<b>Exploration</b>					
Latency to move	- 0.092	0.9265	- 0.012	0.01667	NS
Latency to contact	1.107	0.2683	0.139	0.00357	NS
Latency to rotate	1.067	0.2861	0.139	0.00385	NS
Latency to insert chelipeds	0.560	0.5755	0.074	0.00833	NS
Contact duration	0.012	0.9904	0.002	0.05000	NS
Rotation duration	0.617	0.5374	0.081	0.00625	NS
Cheliped insertion duration	- 0.563	0.5733	- 0.074	0.00714	NS
Total investigation duration	- 0.751	0.4526	- 0.094	0.00556	NS
<b>Aggression</b>					
Presentation duration	- 1.536	0.1246	- 0.190	0.00313	NS
Extension duration	- 1.985	0.0471	- 0.243	0.00263	NS
Ambulatory raise duration	- 1.914	0.0556	- 0.235	0.00278	NS
High posture duration	- 0.858	0.3908	- 0.107	0.00455	NS
Total cheliped displays	- 2.000	0.0455	- 0.245	0.00250	NS
Total walking leg displays	- 1.680	0.0929	- 0.207	0.00294	NS
Total displays duration	- 2.152	0.0314	- 0.263	0.00238	NS
Latency to contact defender	- 0.767	0.4430	- 0.096	0.00500	NS
Latency to insert chelipeds	- 1.367	0.1716	- 0.169	0.00333	NS
Latency to first rap	- 1.009	0.3132	- 0.126	0.00417	NS
Raps/bout	0.047	0.9622	0.006	0.02500	NS
Mean pause duration	- 0.126	0.9001	- 0.016	0.01250	NS

## REFERENCES

- Abdenmour, C. 1997: Copper, zinc and haemocyanin concentrations in four caridean decapods (Crustacea): size relationships. *Hydrobiologia* **346**, 1-9.
- Abrahams, M. V., Robb, T. L. & Hare, J. F. 2005: Effect of hypoxia on opercular displays: evidence for an honest signal? *Animal Behaviour* **70**, 427-432.
- Adams, E. S. & Caldwell, R. L. 1990: Deceptive communication in asymmetric fights of the stomatopod crustacean *Gonodactylus bredini*. *Animal Behaviour* **39**, 706-716.
- Adams, W. C., Bernauer, E. M., Dill, D. B. & Bomar, J. B. 1975: Effects of equivalent sea-level and altitude training on  $VO_2$ max and running performance. *Journal of Applied Physiology* **39**, 262-266.
- Arnott, G. & Elwood, R. W. 2007: Fighting for shells: how private information about resource value changes hermit crab pre-fight displays and escalated fight behaviour. *Proceedings of the Royal Society of London Series B-Biological Sciences* **274**, 3011-3017.
- . 2008: Information gathering and decision making about resource value in animal contests. *Animal Behaviour* **76**, 529-542.
- Arnqvist, G. & Henriksson, S. 1997: Sexual cannibalism in the fishing spider and a model for the evolution of sexual cannibalism based on genetic constraints. *Evolutionary Ecology* **11**, 255-273.
- Athanasopoulos, N. 1994: Flame methods manual for atomic absorption. GBC Scientific equipment PTY LTD, Victoria, Australia.
- Atwood, H. L. 1973: An attempt to account for the diversity of crustacean muscles. *American Zoologist* **13**, 357-378.
- Backwell, P. R. Y., Christy, J. H., Telford, S. R., Jennions, M. D. & Passmore, N. I. 2000: Dishonest signalling in a fiddler crab. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 719-724.
- Baden, S. P., Depledge, M. H. & Hagerman, L. 1994: Glycogen depletion and altered copper and manganese handling in *Nephrops norvegicus* following starvation and exposure to hypoxia. *Marine Ecology Progress Series* **103**, 65-72.
- Barki, A., Harpaz, S. & Karplus, I. 1997: Contradictory asymmetries in body and weapon size, and assessment in fighting male prawns, *Macrobrachium rosenbergii*. *Aggressive Behavior* **23**, 81-91.
- Bee, M. A., Perrill, S. A. & Owen, P. C. 2000: Male green frogs lower the pitch of acoustic signals in defense of territories: a possible dishonest signal of size? *Behavioral Ecology* **11**, 169-177.
- Bell, A. M. 2007: Future directions in behavioural syndromes research. *Proceedings of the Royal Society of London Series B-Biological Sciences* **274**, 755-761.
- Boardman, D. L. & Collier, H. O. J. 1946: The effect of magnesium deficiency on neuromuscular transmission in the shore crab, *Carcinus maenas*. *Journal of Physiology* **104**, 377-383.
- Booth, C. E., McMahon, B. R. & Pinder, A. W. 1982: Oxygen uptake and the potentiating effects of increased hemolymph lactate on oxygen transport during exercise in the blue crab, *Callinectes sapidus*. *Journal of Comparative Physiology B - Biochemical, Systems, and Environmental Physiology* **148**, 111-121.
- Bradford, M. M. 1976: A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* **72**, 248-254.



- Brandt, Y. 2003: Lizard threat display handicaps endurance. *proceedings of the Royal Society of London Series B-Biological Sciences* **270**, 1061-1068.
- Brick, O. & Jakobsson, S. 2002: Individual variation in risk taking: the effect of a predatory threat on fighting behavior in *Nannacara anomala*. *Behavioral Ecology* **13**, 439-442.
- Bridges, C. R. 2001: Modulation of haemocyanin oxygen affinity: Properties and physiological implications in a changing world. *Journal of Experimental Biology* **204**, 1021-1032.
- Briffa, M. 2006: Signal residuals during shell fighting in hermit crabs: can costly signals be used deceptively? *Behavioral Ecology* **17**, 510-514.
- Briffa, M. & Elwood, R. W. 2000a: Analysis of the finescale timing of repeated signals: does shell rapping in hermit crabs signal stamina? *Animal Behaviour* **59**, 159-165.
- . 2000b: Cumulative or sequential assessment during hermit crab shell fights: effects of oxygen on decision rules. *Proceedings of the Royal Society of London Series B-Biological Sciences* **267**, 2445-2452.
- . 2000c: The power of shell rapping influences rates of eviction in hermit crabs. *Behavioral Ecology* **11**, 288-293.
- . 2001: Decision rules, energy metabolism and vigour of hermit-crab fights. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 1841-1848.
- . 2002: Power of shell-rapping signals influences physiological costs and subsequent decisions during hermit crab fights. *Proceedings of the Royal Society of London Series B-Biological Sciences* **269**, 2331-2336.
- . 2004: Use of energy reserves in fighting hermit crabs. *Proceedings of the Royal Society of London Series B-Biological Sciences* **271**, 373-379.
- . 2005: Rapid change in energy status in fighting animals: causes and effects of strategic decisions. *Animal Behaviour* **70**, 119-124.
- . 2007: Monoamines and decision making during contests in the hermit crab *Pagurus bernhardus*. *Animal Behaviour* **73**, 605-612.
- . In press. Difficulties remain in distinguishing between mutual and self assessment in animal contests. *Animal Behaviour*, *In press*.
- Briffa, M., Elwood, R. W. & Dick, J. T. A. 1998: Analysis of repeated signals during shell fights in the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265**, 1467-1474.
- Briffa, M., Haskell, P. & Wilding, C. 2008a: Behavioural colour change in the hermit crab *Pagurus bernhardus*: Reduced crypticity when the threat of predation is high. *Behaviour* **145**, 915-929.
- Briffa, M. & Mowles, S. L. 2008: Hermit Crabs. *Current Biology* **18**, r144-r146.
- Briffa, M., Rundle, S. D. & Fryer, A. 2008b: Comparing the strength of behavioural plasticity and consistency across situations: animal personalities in the hermit crab *Pagurus bernhardus*. *Proceedings of the Royal Society of London Series B-Biological Sciences* **275**.
- Briffa, M. & Sneddon, L. U. 2007: Physiological constraints on contest behaviour. *Functional Ecology* **21**, 627-637.
- Burmeister, S. S., Ophir, A. G., Ryan, M. J. & Wilczynski, W. 2002: Information transfer during cricket frog contests. *Animal Behaviour* **64**, 715-725.
- Caldwell, R. L. & Dingle, J. 1979: Influence of size differential on agonistic encounters in the mantis shrimp, *Gonodactylus viridis*. *Behaviour* **69**, 254-264.
- Cameron, J. N. 1985: Compensation of hypercapnic acidosis in the aquatic blue crab, *Callinectes sapidus*: the predominance of external sea water over carapace carbonate as the proton sink. *Journal of Experimental Biology* **114**, 197-206.

- Chadwick Johnson, J. & Sih, A. 2005: Precopulatory sexual cannibalism in fishing spiders (*Dolomedes triton*): a role for behavioural syndromes. *Behavioral Ecology and Sociobiology* **58**, 390-396.
- . 2007: Fear, food, sex and parental care: a syndrome of boldness in the fishing spider, *Dolomedes triton*. *Animal Behaviour* **74**, 1131-1138.
- Chapple, W. D. 1993: Dynamics of reflex cocontraction in hermit crab abdomen: experiments and a systems model. *Journal of Neurophysiology* **69**, 1904-1917.
- Clobert, J., Oppliger, A., Sorci, G., Ernande, B., Swallow, J. G. & Garland Jr, T. 2000: Trade-offs in phenotypic traits: endurance at birth, growth, survival, predation and susceptibility to parasitism in a lizard, *Lacerta vivipara*. *Functional Ecology* **14**, 675-684.
- Clutton-Brock, T. H. & Albon, S. D. 1979: Roaring of red deer and the evolution of honest advertisement. *Behaviour* **69**, 145-170.
- Clutton-Brock, T. H., Albon, S. D., Gibson, R. M. & Guinness, F. E. 1979: The logical stag - adaptive aspects of fighting in red deer (*Cervus elaphus* L.). *Animal Behaviour* **27**, 211-225.
- Coleman, K. & Wilson, D. S. 1998: Shyness and boldness in pumpkinseed sunfish: individual differences are context specific. *Animal Behaviour* **56**, 927-936.
- Cordero, A. 1999: Forced copulations and female contact guarding at a high male density in a calopterygid damselfly. *Journal of Insect Biology* **12**, 27-37.
- Cornell, J. C. 1979: Salt and water balance in two marine spider crabs, *Libinia emarginata* and *Pugettia producta*. 1. Urine production and magnesium regulation. *The Biological Bulletin* **157**, 221-233.
- Dall, S. R. X., Houston, A. I. & McNamara, J. M. 2004: The behavioural ecology of personality: consistent individual differences from an adaptive perspective. *Ecology Letters* **7**, 734-739.
- Dawkins, M. S. & Guilford, T. 1991: The corruption of honest signalling. *Animal Behaviour* **41**, 865-873.
- Debelius, H. 2001: Crustacea Guide of the World. IKAN - Unterwasserarchiv, Frankfurt, Germany.
- DeCarvalho, T. N., Watson, P. J. & Field, S. A. 2004: Costs increase as ritualized fighting progresses within and between phases in the sierra dome spider, *Neriene litigiosa*. *Animal Behaviour* **68**, 473-482.
- DeFur, P. L., Mangum, C. P. & Reese, J. E. 1990: Respiratory responses of the blue crab *Callinectes sapidus* to long-term hypoxia. *Biological Bulletin* **178**, 46-54.
- DeFur, P. L., Wilkes, P. R. H. & McMahon, B. R. 1980: Non-equilibrium acid-base status in *Cancer productus*: Role of exoskeletal buffers. *Respiratory Physiology* **42**, 247-261.
- Dick, J. T. A. & Elwood, R. W. 1996: Effects of natural variation in sex ratio and habitat structure on mate guarding decisions in amphipods (Crustacea). *Behaviour* **133**, 985-996.
- Dowds, B. M. & Elwood, R. W. 1983: Shell wars: assessment strategies and the timing of decisions in hermit crab shell fights. *Behaviour* **85**, 1-24.
- Duckworth, R. A. 2006: Behavioural correlations across breeding contexts provide a mechanism for a cost of aggression. *Behavioral Ecology* **17**, 1011-1019.
- Elwood, R. W. & Briffa, M. 2001: Information gathering and communication during agonistic encounters: A case study of hermit crabs. In: *Advances in the Study of Behavior*, Vol 30. pp. 53-97.
- Elwood, R. W. & Glass, C. W. 1981: Negotiation or aggression during shell fights in the hermit crab *Pagurus bernhardus*. *Animal Behaviour* **29**, 1239-1244.

- Elwood, R. W. & Neil, S. J. 1992: Assessments and Decisions: A Study of Information Gathering by Hermit Crabs. Chapman and Hall, London.
- Elwood, R. W., Wood, K. E., Gallagher, M. B. & Dick, J. T. A. 1998: Probing motivational state during agonistic encounters in animals. *Nature* **393**, 66-68.
- Elwood, R. W., Pothanikat, R. M. E. & Briffa, M. 2006: Honest and dishonest displays, motivational state and subsequent decisions in hermit crab shell fights. *Animal Behaviour* **72**, 853-859.
- Enquist, M. 1985: Communication during aggressive interactions with special reference to variation in choice of behaviour. *Animal Behaviour* **33**, 1152-1161.
- Enquist, M. & Leimar, O. 1983: Evolution of fighting behavior - decision rules and assessment of relative strength. *Journal of Theoretical Biology* **102**, 387-410.
- Enquist, M., Leimar, O., Ljungberg, T., Mallner, Y. & Segerdahl, N. 1990: A test of the sequential assessment game - fighting in the cichlid fish *Nannacara anomala*. *Animal Behaviour* **40**, 1-14.
- Fabiato, A. & Fabiato, F. 1975: Effects of magnesium on contractile activation of skinned cardiac cells. *Journal of Physiology-London* **249**, 497-517.
- Fahrenbach, W. H. 1967: The fine structure of fast and slow crustacean muscles. *Journal of Cell Biology* **35**, 69-79.
- Frederich, M., DeWachter, B., Sartoris, F. J. & Portner, H. O. 2000a: Cold tolerance and the regulation of cardiac performance and hemolymph distribution in *Maja squinado* (Crustacea : Decapoda). *Physiological and Biochemical Zoology* **73**, 406-415.
- Frederich, M., Sartoris, F. J., Arntz, W. E. & Portner, H. O. 2000b: Haemolymph  $Mg^{2+}$  regulation in decapod crustaceans: Physiological correlates and ecological consequences in polar areas. *Journal of Experimental Biology* **203**, 1383-1393.
- Frederich, M., Sartoris, F. J. & Portner, H. O. 2001: Distribution patterns of decapod crustaceans in polar areas: a result of magnesium regulation? *Polar Biology* **24**, 719-723.
- Gammell, M. P. & Hardy, I. C. W. 2003: Contest duration: sizing up the opposition? *Trends in Ecology and Evolution* **18**, 491-493.
- Geist, V. 1974: Fighting strategies in animal combat. *Nature* **250**, 354-354.
- Gherardi, F. 1996: Non-conventional hermit crabs: pros and cons of a sessile, tube-dwelling life in *Discorsopagurus schmitti* (Stevens). *Journal of Experimental Marine Biology and Ecology* **202**, 119-136.
- . 2004: Resource partitioning between sexes in the "unconventional" hermit crab, *Calcinus tubularis*. *Behavioral Ecology* **15**, 742-747.
- Gosling, S. D. 2001: From mice to men: what can we learn about personality from animal research? *Psychological Bulletin* **127**, 45-86.
- Grafen, A. 1990: Biological signals as handicaps. *Journal of Theoretical Biology* **144**, 517-546.
- Hack, M. A. 1997: The energetic costs of fighting in the house cricket, *Acheta domesticus* L. *Behavioural Ecology* **8**, 28-36.
- Hagerman, L. 1986: Haemocyanin concentration in the shrimp *Crangon crangon* (L.) after exposure to moderate hypoxia. *Comparative Biochemistry and Physiology a-Molecular and Integrative Physiology* **85**, 721-724.
- Hamilton, W. D. 1971: Geometry for the selfish herd. *Journal of Theoretical Biology* **31**, 295-311.
- Hammerstein, P. & Parker, G. A. 1982: The asymmetric war of attrition. *Journal of Theoretical Biology* **96**, 647-682.
- Hazlett, B. A. 1978: Shell exchanges in hermit crabs: aggression, negotiation or both? *Animal Behaviour* **26**, 1278-1279.

- . 1987: Information transfer during shell exchanges in the hermit crab *Calcinus tibicen*. *Animal Behaviour* **35**, 218-226.
- . 1995: Behavioural plasticity in crustacea: why not more? *Journal of Experimental Marine Biology and Ecology* **193**, 57-66.
- . 1996: Assessments during shell exchanges by the hermit crab *Clibanarius vittatus*: The complete negotiator. *Animal Behaviour* **51**, 567-573.
- Heinicke, K., Heinicke, I., Schmidt, W. & Wolfarth, B. 2005: A three-week traditional altitude training increases hemoglobin mass and red cell volume in elite biathlon athletes. *International Journal of Sports Medicine* **26**, 350-355.
- Henry, R. P., Kormanik, G. A., Smatresk, N. J. & Cameron, J. N. 1981a: The role of  $\text{CaCO}_3$  dissolution as a source of  $\text{HCO}_3^-$  for the buffering of hypercapnic acidosis in aquatic and terrestrial decapod crustaceans. *Journal of Experimental Biology* **94**, 269-274.
- Herrel, A., Spithoven, L., Van Damme, R. & De Vree, F. 1999: Sexual dimorphism of head size in *Gallotia galloti*: testing the niche divergence hypothesis by functional analysis. *Functional Ecology* **13**, 289-297.
- Holliday, C. W. 1980: Magnesium transport by the urinary bladder of the crab, *Cancer magister*. *Journal of Experimental Biology* **85**, 187-201.
- Horn, A. G., Leonard, M. L. & Weary, D. M. 1995: Oxygen consumption during crowing by roosters: talk is cheap. *Animal Behaviour* **50**, 1171-1175.
- Hughes, M. 2000: Deception with honest signals: signal residuals and signal function in snapping shrimp. *Behavioral Ecology* **11**, 614-623.
- Huntingford, F. A. 1976: The relationship between anti-predator behaviour and aggression among conspecifics in the three-spined stickleback. *Animal Behaviour* **24**, 245-260.
- Hurd, P. L. 1997: Is signalling of fighting ability costlier for weaker individuals? *Journal of Theoretical Biology* **184**, 83-88.
- Husak, J. F., Irschick, D. J., Meyers, J. J., Lailvaux, S. P. & Moore, I. T. 2007: Hormones, sexual signals, and performance of anole lizards (*Anolis carolinensis*). *Hormones and Behavior* **52**, 360-367.
- Huxley, J. S. 1966: Introduction. In J. S. Huxley, ed., *A discussion on ritualization of behaviour in animals and man*. *Philosophical Transactions of the Royal Society of London, Series B: Biological Sciences* **251**, 249-271.
- Huyghe, K., Vanhooydonck, B., Scheers, H., Molina-Borja, M. & Van Damme, R. 2005: Morphology, performance and fighting capacity in male lizards, *Gallotia galloti*. *Functional Ecology* **19**, 800-807.
- Hyman, J. & Hughes, M. 2006: Territory owners discriminate between aggressive and nonaggressive neighbours. *Animal Behaviour* **72**, 209-215.
- Irschick, D. J. & Garland Jr, T. 2001: Integrating function and ecology in studies of adaptation: Investigations of locomotor capacity as a model system. *Annual Review of Ecology and Systematics* **32**, 367-396.
- Jackson, D. C. 2000: How a turtle's shell helps it survive prolonged anoxic acidosis. *News in Physiological Sciences* **15**, 181-185.
- Jackson, N. W. & Elwood, R. W. 1989: How animals make assessments: information gathering by the hermit crab *Pagurus bernhardus*. *Animal Behaviour* **38**, 951-957.
- Jackson, N. W. & Elwood, R. W. 1990: Interrupting an assessment process to probe changes in motivational state. *Animal Behaviour* **39**, 1068-1077.
- Ketterson, E. D. & Nolan, V. 1999: Adaptation, exaptation, and constraint: a hormonal perspective. *American Naturalist* **154**, S4-S25.
- Kobayashi, M. & Gono, H. 1985: Horizontal movement of pale and red *Daphnia magna* in low oxygen concentration. *Physiological Zoology* **58**, 190-196.

- Koolhaas, J. M., Korte, S. M., De Boer, S. F., Van Der Vegt, B. J., Van Reenen, C. G., Hopster, H., De Jong, I. C., Ruis, M. A. W. & Blokhuis, H. J. 1999: Coping styles in animals: current status in behavior and stress-physiology. *Neuroscience and Biobehavioral Reviews* **23**, 925-935.
- Laidre, M. E. & Elwood, R. W. 2008: Motivation matters: cheliped extension displays in the hermit crab, *Pagurus bernhardus*, are honest signals of hunger. *Animal Behaviour* **75**, 2041-2047.
- Lailvaux, S. P., Hathway, J., Pomfret, J. & Knell, R. J. 2005: Horn size predicts physical performance in the beetle *Euoniticellus intermedius* (Coleoptera : Scarabaeidae). *Functional Ecology* **19**, 632-639.
- Lailvaux, S. P., Herrel, A., VanHooydonck, B., Meyers, J. J. & Irschick, D. J. 2004: Performance capacity, fighting tactics and the evolution of life-stage male morphs in the green anole lizard (*Anolis carolinensis*). *Proceedings of the Royal Society of London Series B - Biological Sciences* **271**, 2501-2508.
- Lailvaux, S. P. & Irschick, D. 2007: The evolution of performance-based male fighting ability in Caribbean *Anolis* lizards. *The American Naturalist* **170**, 573-586.
- Leal, M. 1999: Honest signalling during prey-predator interactions in the lizard *Anolis cristatellus*. *Animal Behaviour* **58**, 521-526.
- Leimar, O., Auatad, S. & Enquist, M. 1991: A test of the sequential assessment game – fighting in the bowl and doily spider *Frontinella pyramitela*. *Evolution* **45**, 862-874.
- Leimar, O. & Enquist, M. 1984: Effects of asymmetries in owner-intruder conflicts. *Journal of Theoretical Biology* **111**, 475-491.
- Lewontin, R. C. 1961: Evolution and the theory of games. *Journal of Theoretical Biology* **1**, 382-403.
- Lohuis, T. D., Harlow, H. J. & Beck, T. D. I. 2007: Hibernating black bears (*Ursus americanus*) experience skeletal muscle protein during winter anorexia. *Comparative Biochemistry and Physiology* **147**, 20-28.
- Mangum, C. P. 1990: Recent advances in hemocyanin physiology. In: *Invertebrate dioxygen carriers*. (Preaux, G. & Lontie, R., eds). Leuven University Press, Leuven, Belgium. pp. 449-459.
- . 1997: Invertebrate blood oxygen carriers. In: *Handbook of Physiology, Section 13, Comparative Physiology*. (Dantzler, W. H., ed). Oxford University Press, New York. pp. 1097-1135.
- Maynard Smith, J. 1974: Theory of games and evolution of animal conflicts. *Journal of Theoretical Biology* **47**, 209-221.
- Maynard Smith, J. & Parker, G. A. 1976: Logic of asymmetric contests. *Animal Behaviour* **24**, 159-175.
- Maynard Smith, J. & Price, G. R. 1973: The logic of animal conflict. *Nature* **246**, 15-18.
- McAllen, R., Taylor, A. & Freel, J. 2005: Seasonal variation in the ionic and protein content of haemolymph from seven deep-sea decapod genera from the Northeast Atlantic Ocean. *Deep-Sea Research Part I-Oceanographic Research Papers* **52**, 2017-2028.
- Molina-Borja, M., Padron-Fumero, M. & Afonso-Martin, T. 1998: Morphological and behavioural traits affecting the intensity and outcome of male contests in *Gallotia galloti galloti* (Family Lacertidae). *Ethology* **104**, 314-322.
- Morritt, D. & Spicer, J. I. 1993: A brief re-examination of the function and regulation of extracellular magnesium and its relationship to activity in crustacean arthropods. *Comparative Biochemistry and Physiology a-Physiology* **106**, 19-23.
- Mowles, S. L., Briffa, M., Cotton, P. A. & Spicer, J. I. 2008: The role of circulating metal ions during shell fights in the hermit crab *Pagurus bernhardus*. *Ethology* **114**, 1014-1022.

- Nash, J. F. 1951: Non-cooperative games. *Annals of Mathematics* **54**, 286-295.
- Neat, F. C., Taylor, A. C. & Huntingford, F. A. 1998: Proximate costs of fighting in male cichlid fish: the role of injuries and energy metabolism. *Animal Behaviour* **55**, 875-882.
- Neil, S. J. & Elwood, R. W. 1985: Behavioral modifications during egg-brooding in the hermit crab, *Pagurus bernhardus* L. *Journal of Experimental Marine Biology and Ecology* **94**, 99-114.
- Neumann, J. v. & Morgenstern, O. 1944: Theory of games and economic behaviour. Princeton Univeristy Press., Princeton.
- Nickerson, K. W. & Van Holde, K. E. 1971: A comparison of molluscan and arthropod haemocyanin - I. Circular dichromism and absorption spectra. *Comparative Biochemistry and Physiology* **39B**, 855-872.
- Nies, A., Zeis, B., Bridges, C. R. & Grieshaber, M. K. 1992: Allosteric modulation of haemocyanin oxygen-affinity by *L*-lactate and urate in the lobster *Homarus vulgaris* : II. Characterization of specific effector binding sites *Journal of Experimental Biology* **168**, 111-124.
- Ogonowski, M. M. & Lang, F. 1979: Histochemical evidence for enzyme differences in crustacean fast and slow muscle. *Journal of Experimental Zoology* **207**, 143-151.
- Parker, G. A. 1974: Assessment strategy and evolution of fighting behavior. *Journal of Theoretical Biology* **47**, 223-243.
- Payne, R. J. H. 1998: Gradually escalating fights and displays: the cumulative assessment model. *Animal Behaviour* **56**, 651-662.
- Payne, R. J. H. & Pagel, M. 1996: Escalation and time costs in displays of endurance. *Journal of Theoretical Biology* **183**, 185-193.
- . 1997: Why do animals repeat displays? *Animal Behaviour* **54**, 109-119.
- Perry, G., Levering, K., Girard, I. & Garland, T. J. 2004: Locomotor performance and social dominance in male *Anolis cristatellus*. *Animal Behaviour* **67**, 37-47.
- Purves, W. K., Sadava, D., Orians, G. H. & Heller, H. C. 2001: Life, the Science of Biology, 6th edn., 6 edn. Sinauer Associates, Inc., Sunderland, MA.
- Rands, S. A., Cowlshaw, G., Pettifor, R. A., Rowcliffe, J. M. & Johnstone, R. A. 2003: Spontaneous emergence of leaders and followers in foraging pairs. *Nature* **423**, 432-434.
- Réale, D., Gallant, B. Y., Leblanc, M. & Festa-Bianchet, M. 2000: Consistency of temperament in bighorn ewes and correlates with behaviour and life history. *Animal Behaviour* **60**, 589-597.
- Reaney, L. T. & Backwell, P. R. Y. 2007: Risk-taking behavior predicts aggression and mating success in a fiddler crab. *Behavioral Ecology* **18**, 521-525.
- Redfield, A. C. 1934: The haemocyanins. *Biological Reviews* **9**, 175-212.
- Redmond, J. R. 1955: The respiratory function of hemocyanin in crustacea. *Journal of Cellular and Comparative Physiology* **46**, 209-247.
- Riechert, S. E. 1978: Games spiders play - Behavioral variability in territorial disputes. *Behavioral Ecology and Sociobiology* **3**, 135-162.
- Riechert, S. E. & Hedrick, A. V. 1993: A test for correlations among fitness-linked behavioural traits in the spider *Agelenopsis aperta*. *Animal Behaviour* **46**, 669-675.
- Riechert, S. E. & Maynard Smith, J. 1989: Genetic analyses of two behavioural traits linked to individual fitness in the desert spider *Agelenopsis aperta*. *Animal Behaviour* **37**, 624-637.
- Robertson, J. D. 1949: Ionic regulation in some marine invertebrates. *Journal of Experimental Biology* **26**, 182-200.

- Robson, M. A. & Miles, D. B. 2000: Locomotor performance and dominance in male tree lizards, *Urosaurus ornatus*. *Functional Ecology* **14**, 338-344.
- Rotjan, R., Blum, J. & Lewis, S. 2004: Shell choice in *Pagurus longicarpus* hermit crabs: does predation threat influence shell selection behaviour? *Behavioral Ecology and Sociobiology* **56**, 171-176.
- Sadava, D., Heller, H. C., Orians, G. H., Purves, W. K. & Hillis, D. M. 2008: *Life, the Science of Biology*, 8th edn. Sinauer Associates, Inc., Sunderland, MA.
- Schuett, G. W. & Grober, M. S. 2000: Post-fight levels of plasma lactate and corticosterone in male copperheads, *Agkistrodon contortrix* (Serpentes, Viperidae): differences between winners and losers. *Physiology & Behavior* **71**, 335-341.
- Shuster, S. M. & Caldwell, R. L. 1982: Cavity defense by pairs in the stomatopod, *Gonodactylus bredini*. *American Zoologist* **22**, 973.
- Sigel, H. & Sigel, A. 1990: *Metal Ions in Biological Systems. Volume 26: Compendium on Magnesium and its Role in Biology, Nutrition, and Physiology*. Marcel Dekker, Inc., New York.
- Sih, A., Bell, A. & Chadwick Johnson, J. 2004: Behavioral syndromes: an ecological and evolutionary overview. *Trends in Ecology and Evolution* **19**, 372-378.
- Sih, A., Kats, L. B. & Maurer, E. F. 2003: Behavioural correlations across situations and the evolution of antipredator behaviour in a sunfish-salamander system. *Animal Behaviour* **65**, 29-44.
- Sinervo, B., Miles, D. B., Frankino, A. W., Klukowski, M. & DeNardo, D. F. 2000: Testosterone, endurance, and Darwinian fitness: Natural and sexual selection on the physiological bases of alternative male behaviours in side-blotched lizards. *Hormones and Behaviour* **38**, 222-233.
- Sinn, D. L., Gosling, S. D. & Moltschaniwskyj, N. A. 2008: Development of shy/bold behaviour in squid: context-specific phenotypes associated with developmental plasticity. *Animal Behaviour* **75**, 433-442.
- Sloan Wilson, D., Coleman, K., Clark, A. B. & Biederman, L. 1993: hy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a physiological trait. *Journal of Comparative Psychology* **107**, 250-260.
- Smallegange, I. M., Sabelis, M. W. & van der Meer, J. 2007: Assessment games in shore crab fights. *Journal of Experimental Marine Biology and Ecology* **351**, 255-266.
- Sneddon, L. U. 2003: The bold and the shy: individual differences in rainbow trout. *Journal of Fish Biology* **62**, 971-975.
- Sneddon, L. U., Huntingford, F. A. & Taylor, A. C. 1997: Weapon size versus body size as a predictor of winning in fights between shore crabs, *Carcinus maenas* (L.). *Behavioral Ecology and Sociobiology* **41**, 237-242.
- Sneddon, L. U., Huntingford, F. A., Taylor, A. C. & Orr, J. F. 2000a: Weapon strength and competitive success in the fights of shore crabs (*Carcinus maenas*). *Journal of Zoology* **250**, 397-403.
- Sneddon, L. U., Taylor, A. C. & Huntingford, F. A. 1999: Metabolic consequences of agonistic behaviour: Crab fights in declining oxygen tensions. *Animal Behaviour* **57**, 353-363.
- Sneddon, L. U., Taylor, A. C., Huntingford, F. A. & Watson, D. G. 2000b: Agonistic behaviour and biogenic amines in shore crabs *Carcinus maenas*. *Journal of Experimental Biology* **203**, 537-545.
- Spicer, J. I. & Baden, S. P. 2000: Natural variation in the concentrations of haemocyanin from three decapod crustaceans, *Nephrops norvegicus*, *Liocarcinus depurator* and *Hyas araneus*. *Marine Biology* **136**, 55-61.

- . 2001: Environmental hypoxia and haemocyanin between-individual variability in Norway lobsters *Nephrops norvegicus* (L.). *Marine Biology* **139**, 727-734.
- Spicer, J. I., Morritt, D. & Taylor, A. C. 1994: Effect of low temperature on oxygen uptake and haemolymph ions in the sandhopper *Talitrus saltator* (Crustacea Amphipoda). *Journal of the Marine Biological Association of the United Kingdom* **74**, 313-321.
- Steger, R. & Caldwell, R. L. 1983: Intraspecific deception by bluffing: A defence strategy of newly molted stomatopods (Arthropoda: Crustacea). *Science* **221**, 558-560.
- Sullivan, B. K. & Walsberg, G. E. 1985: Call rate and aerobic capacity in woodhouse's toad (*Bufo woodhousei*). *Herpetologica* **41**, 404-407.
- Számadó, S. 2008: How threat displays work: species-specific fighting techniques, weaponry and proximity risk. *Animal Behaviour* **76**, 1455-1463.
- Taylor, P. W. & Elwood, R. W. 2003: The mismeasure of animal contests. *Animal Behaviour* **65**, 1195-1202.
- Terwilliger, N. B. 1998a: Functional adaptations of oxygen-transport proteins. *Journal of Experimental Biology* **201**, 1085-1098.
- Thornhill, R. 1984: Fighting and assessment in *Harpobittacus* scorpionflies. *Evolution* **38**, 204-214.
- Thorpe, K. E., Taylor, A. C. & Huntingford, F. A. 1995: How costly is fighting? Physiological effects of sustained exercise and fighting in swimming crabs, *Necora puber* (L.) (Brachyura, Portunidae). *Animal Behaviour* **50**, 1657-1666.
- Truchot, J. P. 1975: Factors controlling the *in vitro* and *in vivo* oxygen affinity of the haemocyanin in the crab *Carcinus maenas* (L.). *Respiration Physiology* **24**, 173-189.
- Truchot, J. P. & Lallier, F. H. 1992: Modulation of the oxygen-carrying function of haemocyanin in crustaceans. *News in Physiological Sciences* **7**, 49-52.
- U.S.E.P.A. 1983: Calcium: Method 215.1 (atomic absorption, direct aspiration). In: *Methods for chemical analysis of water and wastes*, EPA-600/4-79-020. U.S. Environmental Protection Agency, Cincinnati, Ohio, USA.
- Vanhooydonck, B., Herrel, A. Y., Van Damme, R. & Irschick, D. J. 2005: Does dewlap size predict male bite performance in Jamaican *Anolis* lizards? *Functional Ecology* **19**, 38-42.
- Verrell, P. A. 1986: Wrestling in the red-spotted newt (*Notophthalmus viridescens*): resource value and contestant asymmetry determine contest duration and outcome. *Animal Behaviour* **34**, 398-402.
- Vye, C., Cobb, J. S., Bradley, T., Gabbay, J., Genizi, A. & Karplus, I. 1997: Predicting the winning or losing of symmetrical contests in the American lobster *Homarus americanus* (Milne-Edwards). *Journal of Experimental Marine Biology and Ecology* **217**, 19-29.
- Wagner, W. E. 1992: Deceptive or honest signalling of fighting ability - a test of alternative hypotheses for the function of changes in call dominant frequency by male cricket frogs. *Animal Behaviour* **44**, 449-462.
- Walters, N. J. & Uglow, R. F. 1981: Haemolymph magnesium and relative heart activity of some species of marine decapod crustaceans. *Journal of Experimental Marine Biology and Ecology* **55**, 255-265.
- Weary, D. M., Lambrechts, M. M. & Krebs, J. R. 1991: Does singing exhaust male great tits? *Animal Behaviour* **41**, 540-542.
- Weinstein, R. B. & Full, R. J. 2000: Intermittent locomotor behaviour alters total work. In: *Experimental Biology Reviews: Biomechanics in Animal Behaviour*. (Domenici, P. & Blake, R. W., eds). BIOS Scientific Publishers Ltd. pp. 33-48.
- Wilkinson & Shank. 1977: Rutting-fight mortality among musk oxen on Banks Island, Northwest Territories, Canada. *Animal Behaviour* **24**, 756-758.



- Wilson, D. S. 1998: Adaptive individual differences within single populations. *Philosophical Transactions of the Royal Society of London Series B - Biological Sciences* **353**, 199-205.
- Wilson, D. S., Coleman, K., Clark, A. B. & Biederman, L. 1993: Shy-bold continuum in pumpkinseed sunfish (*Lepomis gibbosus*): an ecological study of a physiological trait. *Journal of Comparative Psychology* **107**, 250-260.
- Zahavi, A. 1977: Reliability in communication systems and the evolution of altruism. In: *Evolutionary Ecology*. (Stonehouse, B. & Perrins, C., eds). The Macmillan Press, London. pp. 253-259.